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Methods to measure biomass and production of bacteria and photosynthetic microbiota and their application on illuminated lake sediments

A literature study

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Summary

The Swedish Nuclear Fuel and Waste Management Co (SKB) is responsible for the management and disposal of radioactive waste in Sweden. In the work of finding a place for long time storage of radioactive waste it is of importance to understand the surrounding ecosystems. The storage is supposed to keep the radioactive waste away from humans and nature for some hundreds of thousands of years. It is important to be able to make risk assessments for a hypothetical release and understand by which ways the radionuclides could find their way into the biota. In lakes, released radionuclides would most probably find their way into the biota through heterotrophic bacteria or autotrophic microorganisms. Therefore, it is important to investigate how large the biomass and production of heterotrophic bacteria and photosynthetic organisms in lakes are.

This report is an overview of methods that are commonly used today for measuring biomass and production of bacteria and photosynthetic microorganisms in lakes. It elucidates advantages and drawbacks of the different methods. Some results from studies on illuminated lake sediment habitats are given.

Biomass of bacteria is commonly measured in microscope after colouring the bacteria with a dye. Dyes commonly used are acridine orange and 4',6-diamino-2-phenylidole (DAPI). Biomass of photosynthetic microorganisms is also commonly measured in microscope but can also be determined by the amount of chlorophyll *a* and other pigments. An advantage with measuring the biomass photosynthetic microorganisms in microscope is that a good resolution of the community is achieved. A disadvantage with determining the biomass by measuring the chlorophyll a concentrations is that the concentrations may vary with light climate and nutrients even though the carbon biomass is constant.

Methods for measuring bacterial production discussed in this report are the thymidine incorporation method, the leucine incorporation method and the frequency of dividing cell method (FDC). Methods for primary production discussed in this report are the $^{14}CO_2$-incorporation method, the O_2 production method, the O_2-microelectrodes method, the changes in dissolved inorganic carbon method, and the photosynthetic parameters method.

Studies on illuminated lake bottom habitats indicate that biomass and production of benthic heterotrophic bacteria is higher than the biomass and production of pelagic heterotrophic bacteria. Also, the biomass and production of benthic photosynthesising microorganism may be higher than the biomass and production of phytoplankton. Therefore it is important to study benthic as well as pelagic microorgansims when studying lake ecosystems.

Sammanfattning

Svensk Kärnbränslehantering AB (SKB) är ansvariga för hanteringen av svenskt kärnbränsleavfall. När man planerar var ett långtidsförvar av radioaktivt avfall ska förläggas är det viktigt att förstå hur de omkringliggande ekosystemen fungerar. Det radioaktiva avfallet ska förvaras oåtkomligt för människor och natur i hundratusentals år. Det är ändå viktigt att veta hur de omkringliggande ekosystemen fungerar för att kunna göra riskanalyser för människor ifall radionuklider trots allt skulle komma ut i naturen. I sjöar skulle radionuklider kunna finna sin väg in i näringskedjan via heterotrofa bakterier eller autotrofa mikroorganismer. Därför är det viktigt att mäta biomassa och produktion av heterotrofa bakterier och fotosyntetiserande mikroorganismer.

Den här rapporten ger en överblick över vilka metoder som används idag för att mäta biomassor och produktion av bakterier och fotosyntetiserande mikroorganismer. Fördelar och nackdelar med de olika metoderna diskuteras. Dessutom ges resultat från studier på solbelysta mjukbottnar.

Bakteriebiomassor räknas vanligen i mikroskop efter infärgning av bakterierna. Vanliga infärgningsmedel är 4',6-diamino-2-phenylidole (DAPI) eller acridine orange. Idag har även flera nya färger börjat användas för att kunna skilja mellan levande och döda, aktiva och inaktiva celler.

Biomassan hos fotosyntetiserande mikroorganismer kan bestämmas genom räkning i mikroskop eller genom bestämning av halten klorofyll *a* eller andra pigment. Räkning i mikroskop ger även en bra översikt över vilka organismer som finns. En nackdel med att bestämma koncentrationen klorofyll *a* är att den kan variera med näringsstatus och ljusförhållanden medan kolbiomassan är konstant.

Metoder för att mäta bakterieproduktion som diskuteras i denna rapport är tymidin inkorporering, leucin inkorporering och frekvens av delande celler (FDC). Tymidin inkorporering och leucin inkorporering är de metoder som används mest. Det finns ingen bestämd omräkningsfaktor mellan frekvensen delande celler och tillväxthastighet och därför används FDC mera sällan.

Metoder för primärproduktionsmätning som diskuteras är $^{14}CO_2$-inkorporering, O_2-produktion, O_2-mikroelektroder, förändring i koncentrationen löst oorganiskt kol, och fotosyntetiska parametrar. De metoder som oftast används är $^{14}CO_2$-inkorporering och O_2-produktion.

Studier från solbelysta mjukbottnar visar att biomassan och produktionen av bakterier och fotosyntetiserande mikroorganismer på bottnar (bentisk produktion) kan överstiga biomassan och produktionen i den fria vattenmassan (pelagisk produktion). Därför är det viktigt att studera produktion av såväl bentiska som pelagiska bakterier och fotosyntetiserande mikroorganismer.

Contents

1 Introduction

The Swedish Nuclear and Waste Management Co (SKB) is responsible for the management and disposal of radioactive waste in Sweden. SKB is planning repositories for high level radioactive waste. The purpose for the repositories is to keep the radioactive waste away from man and nature in some hundreds of thousands of years. However, it is of importance to know how the radioactive nuclides would react if they would find their way out in the nature during this time span. It is of interest to know where in the biota they may find their way to higher trophic levels such as man. One of the important ecosystems to understand is the lake and the coastal areas since pollutants often find their way out in the nature via the groundwater into lakes and seas.

If radionuclides enter into a lake they can be incorporated into the food web by microbial organisms via primary production or heterotrophic bacterial production. Therefore it is of importance to investigate the bacterial and primary production in the lakes and coastal areas where repositories are planned. By understanding these processes it is possible to make risk assessments in case of a radioactive release. With a good knowledge about the production one can estimate how much radionuclides would be incorporated into the biota in case of a hypothetical release.

There are several methods for measuring bacterial and primary production in water, sediment and on macrophytes (epiphytes). Both *in situ* (in the lake) and *ex situ* (laboratory experiment) methods have been developed. When the aim is to assess the production within a lake, it is better to use *in situ* methods since it better reflects the real conditions. When measuring the production in laboratory a lot of factors are different from the ones experienced in the lake. The substrate, nutrient and light conditions as well as the flow of water is changed. There may also be factors that the researcher is not aware of that are important for the size of the production. Therefore, *ex situ* measurements should be restricted to experimental questions. Then they are sometimes to prefer before the *in situ* measurements since everything except for the experimental question can be kept constant. Most methods for measuring production have initially been applied for water production analysis and have then been further developed to fit also sedimentary and epiphytic conditions. Some methods for microbial production do not separate between heterotrophic bacterial production and primary production. To get a better understanding of the microorganism community it is favourable if these two groups can be separated. This report aim to give an overview of methods that are commonly used today for measuring biomass and production of bacteria and photosynthetic organisms, and to elucidate advantages and drawbacks of the different methods.

2 Sampling

When sampling bacteria and algae it is important to decide whether qualitative or quantitative samples are required. Also, depending on what analyses to be done the samples should be treated different. Biomass samples should be killed and preserved with e.g. Lugols solution so the initial concentration of algae and bacteria can be analysed /Olrik et al., 1996/. For production analysis it is important to keep the samples alive and disturbed as little as possible. Different methods to obtain samples are used for water, sediments and epiphytes.

2.1 Water sampling

Water samples can be taken directly in a bottle. However, microorganisms may be distributed heterogeneously in the water both horizontally and vertically. Thus, it is preferred if integrated water samples are taken. Different amounts of water should be taken from different depths in proportion to the total volume that each depth interval represents in the lake and then mixed to one water sample /Blomqvist, 1995/. It is most convenient to use a long core sampler, e.g. Ramberg sampler, which is possible to close under the water surface, in order to sample from different depths.

2.2 Sediment sampling

Lake bottoms are often characterised as either hard bottoms or soft bottoms. Sediment from soft bottoms is easily collected with a core sampler, e.g. a Willner sampler. In the Willner sampler a core is lowered into the sediment and a mechanism with a closing bulb creates a pressure, which makes it possible to bring the core to the lake surface with the sediment remaining in the core. In very soft sediments with high water content diving may be required to obtain sediment cores since very soft sediment easily drops from the core. At hard bottoms and in stony sediment, the Willner sampler can not be used since stones can easily break the Plexiglas core. In order to sample algae and bacteria from hard surfaces a brushing syringe may be used /Aloi, 1990/. With this equipment a known area of the surface is brushed and the algae/bacteria are collected in the syringe. Some investigators scrape the surface with a knife but this may destroy some of the algae. Stones may also be brought to the surface to sample the algae by chemical solvents.

Benthic algae and cyanobacteria can be highly stratified in the top mm of sediment and a freeze sampler may be used if high resolution of the microalgae and cyanobacteria are required /Wiltshire, 2000/. Liquid nitrogen is used to freeze the sediment without distortion even on the micrometer scale. This method can be used for biomass estimation only, as production measurements require live algae and bacteria.

When sampling sediments it is good to know if there is any groundwater inflow into the lake and, in that case, where it is situated. Algal biomass has been shown to be significantly higher at ground water inflow sites /Hagertey and Kerfoot, 1998/, and hence, it is important to know where the inflows are to be able to take representative samples for the lake.

2.3 Epiphyte sampling

Epiphytic algae may be sampled by scraping, chemical solvents or by shaking the macrophyte /Aloi, 1990/. The morphology of the macrophyte determines which method is best. Most macrophytes can not stand scraping and parts of the macrophyte can contaminate the sample. Shaking does not remove all epiphytes from the macrophytes. However, often 70–90% are recovered /Aloi, 1990/. With chemical solvents most of the epiphytes is collected but their structure may be destroyed, thus preventing taxonomic identification.

To remove bacteria from macrophytes ultrasound, in a sonicator can be used /Velji and Albright, 1986/. The time for sonication has to be determined for different substrates /Thomas and Wetzel, 1995/.

The biomass of epiphytes is positively correlated with the age of the macrophyte tissue /Burkholder and Wetzel, 1989/. Therefore it is important to take in consideration from where epiphytes are sampled to get comparable results.

3 Biomass

3.1 Bacterial biomass

The bacterial biomass can be very high in water and the planktonic bacterial biomass can make up a large part of the total planktonic biomass /Hessen et al.,1990; Blomqvist et al., 1995/. In water millions of bacterial cells may be found in one cubic centimetre /Horne and Goldman, 1994/. The number of bacteria in sediment does not vary much over the year or between years /Boström et al., 1989/. Usually between 10^8 and 10^{10} cells per cm^3 are found in both fresh and salt-water sediments /Schallenberg et al., 1989/.

Bacterial biomass is commonly measured by direct count in epifluorescence microscope but has occasionally also been measured by the macromolecule components DNA /Paul and Carson, 1984/, protein /Simon and Azam, 1989/, ATP /Karl, 1993/ and lipids /Findlay et al., 1989/.

When counting in epifluorescence microscope different dyes are used to stain bacteria. The dyes commonly used are acridine orange /Hobbie et al., 1977/ and 4',6-diamino-2-phenylidole (DAPI) /Porter and Feigh, 1980/. Acridine orange was the first dye that was successfully used to stain bacteria for counting in fluorescence microscope. It is commonly used both in water and in sediment. The advantages with this dye are that it has been commonly used and is cheap. The disadvantage is that also other things than bacteria may be stained. DAPI binds strongly to DNA and is also a commonly used dye both in water and sediment. DAPI staining improves the visualisation and counting of bacteria and blue green algae less than 1 µm, and it extends the sample storage compared to acridine orange /Porter and Feigh, 1980/. A large part of the bacteria in sediment has been shown to be dead or at least very inactive /Boström and Törnblom, 1990; Zweifel and Hagström, 1995/ and, hence, conventional methods with acridine orange and DAPI severely overestimate the abundance of bacteria /Lantz, 1999/. Lately a number of dyes that aim to colour live or intact cells or dead and damaged cells have been used /Jepras et al., 1995; Lopez-Amoros et al., 1995; Porter et al., 1995; Choi et al., 1996; del Giorgio et al., 1997/. When these dyes are used alone or in combination with each other it is possible to assess the share of active/inactive, dead/alive cells from each other.

When counted in microscope, the size of bacteria can be measured direct in the microscope with a micrometer scale. However, the resolution of the fluorescence microscope is about 1 µm, which is close to the size of many aquatic bacteria. Therefore, another common way to measure the size of the bacteria is from a magnified photograph /Fuhrman, 1981; Lee and Fuhrman, 1987; Coveney and Wetzel, 1988/. This will give a better resolution and the measurement will be more exact than when measured direct in the microscope. Cell volumes are calculated from geometric formulas. Computerised image analysis can also be used in the calculation of bacterial size and volume. This gives good estimates of the bacterial volume but the equipment is expensive. The bacterial biomass may be further converted from volume to carbon using conversion factors. However, there are some considerations concerning the conversion factors from cell volume to cell carbon. Lee and Fuhrman /1987/ and Simon and Azam /1989/ showed that small bacteria tend to have more carbon per volume than large bacteria. Therefore it

is important to choose a conversion factor that is representative for the bacterial population in question.

The best way to view the epiphytic bacteria is through a scanning electron microscope (SEM) but this is time consuming and expensive.

3.2 Biomass of microalgae and cyanobacteria

Biomass of microalgae and cyanobacteria can be determined from direct counts in ordinary light microscope, from autofluorescence microscopy, or from content of chlorophyll *a* (chl *a*) and other pigments. Chl *a* can be measured spectrophotometrically /ISO 10260, 1992; Hansson, 1988/ or by High Performance Liquid Chromatography (HPLC). In water the ratio C:Chl *a* is assumed to be relatively constant but in benthic communities this is not always the case. Gould and Gallagher /1990/ reported C:Chl *a* ratios between 18.7 and 60.4 in a study of benthic diatoms. The chl *a* concentrations does not provide any information of the species composition of the algal community. Using HPLC, other pigments than chl *a* can also be detected but the resolution of diversity is still small. By counting the algae and cyanobacteria in an inverted light microscope a better resolution of the algal community is provided. With a scale in the microscope it is possible to measure the size of the algae, and cyanobacteria and geometric formulas for the different species are used to calculate the volume /Blomqvist and Herliz, 1998; Hillebrand et al.,1999/. Conversion factors are used to calculate biomass from the volumes /Rocha and Duncan, 1985; Blomqvist and Herliz, 1998/. A problem with the counting of algae and cyanobacteria is that different laboratories often get different results even when counting the same set of samples and hence studies are difficult to compare /Niemi et al., 1985/.

In a light microscope microalgae and cyanobacteria less than 5µm can not be distinguished from other particles. Using autofluorescence microscope it is easier to distinguish microorganisms from other particles and hence, it is useful especially in sediment samples. Also, it is easy to distinguish different groups of algae in an autofluorescence microscope since different groups have different pigment compositions and hence have different autofluorescence.

Benthic algal populations can be highly stratified and the most algae and cyanobacteria occur in the top 200 µm /Wiltshire, 2000/. Therefore, the top algal layer may be diluted by mixing when samplers with mm to cm scale are used. If the resolution of the top mm is required, microtechniques should be used. By freezing a core with liquid nitrogen, chl *a* and pigments can be determined with a resolution of 0.1 mm /Wiltshire, 2000/. However, this method is time consuming and not easily applied for routine sampling.

The best way to view the epiphytic algae and cyanobacteria is through a scanning electron microscope (SEM) but this is time consuming and expensive.

4 Heterotrophic bacterial production

Bacteria are dependent on a carbon source either as imported carbon or from autotrophs within the lake, and hence the bacterial production is considered to be secondary production and are called heterotrophic production. The bacterial production can contribute significantly to the secondary production in a lake /Cole et al., 1988/. Cole et al. /1988/ calculated from 70 studies that the heterotrophic bacterial production made up 30% of the water column primary production, was roughly twice as large as the secondary production of macrozooplankton and ranged from 0.1 to 2.4 gC m^{-2} d^{-1}. Heterotrophic bacterial production is usually higher in the sediments than in the overlaying water column calculated on an areal basis /Doremus and Clesceri, 1982/. Heterotrophic bacterial production of epiphytes (bacteria attached to macrophytes) can be very high and has in some cases been shown to exceed the planktonic bacterial production /Theil-Nielsen, 1999/. Bacterial production can be measured in different units. The units that are commonly used are production of carbon per cell, per volume (e.g. 1l water, 1g sediment), per m^2 lake area or per cm^2 macrophyte. Also the time unit used differs, and the commonly used units are per hour, per day or per year. It is important to keep in mind what units are used when comparing different studies with each other. Even though the bacterial biomass is relatively constant /Schallenberg et al., 1989/, the productivity can differ by more than an order of magnitude /Moriarty and Pollard, 1981/. No correlation between bacterial biomass and bacterial production has been found in marine /van Duyl and Kop, 1990/, or in freshwater /Brunberg, 1993/ habitats. The heterotrophic bacterial production can be measured by thymidine incorporation, leucine incorporation and by frequency of dividing cells (FDC). The most common way to measure heterotrophic bacterial production is that by thymidine incorporation but lately leucine incorporation has also become a widely used method. Good correlation between the thymidine and leucine method has been found in pelagic environments /Simon and Azam, 1989; Chin-Leo and Benner, 1991; Kirchman and Hoch, 1988/. In sediments the thymidine method has been shown to sometimes underestimate the heterotrophic production compared to the leucine method /Tuominen, 1995/. The thymidine method has also been shown to give lower values than the leucine method in epiphytic heterotrophic bacterial production and Theil-Nielsen and Søndergaard /1999/ found a 7-fold higher heterotrophic production with the leucine incorporation method. FDC often overestimates the heterotrophic bacterial production compared to the other two methods /Riemann and Bell, 1990; Fallon and Boylen, 1990/.

4.1 Thymidine incorporation

The use of tritiated thymidine ([^{3}H]-thymidine) has become the most commonly used method to measure heterotrophic bacterial production in both water and sediments. [^{3}H]-thymidine is incorporated into the DNA of cells and hence the method measures the increase in bacterial cell numbers. Only actively growing cells synthesise DNA, and hence, non-growing cells should not be marked with [^{3}H]-thymidine above the background values /Robarts and Zoharty, 1993/. Only microorganisms that can transport thymidine through the cell membrane and has the thymidine kinase enzyme can incor-

porate thymidine. During short incubations (less than 1 hour) only heterotrophic bacteria can do this /Robarts and Zoharty, 1993/.

The method appears relatively easy. [^{3}H]-thymidine is added to samples, which are incubated *in situ*. Blanks are prepared by killing the sample before the incubation with e.g. formaldehyde. After the incubation, DNA can be extracted and by measuring the radioactivity the amount of incorporated thymidine can be calculated. The amount of incorporated thymidine for a certain time period and volume of water/sediment/ macrophytes is used together with conversion factors to calculate the production in g C per litre and time. Despite that this procedure seems easy, fundamental knowledge of the thymidine metabolism is needed to prevent serious methodological errors.

In bacterial cells there are two pathways of synthesising the thymidine monophosphate (dTMP) which is used in the synthesis of DNA; the *de novo* pathway and the salvage pathway (Figure 4-1). In the salvage pathway, thymidine is incorporated into the cell and converted by thymidine kinase to dTMP. The dTMP is then converted into thymidine diphosphate (dTDP), further into thymidine triphosphate (dTTP) and finally into DNA. In the *de novo* pathway, nucleotides are synthesised without passing a stage containing free purine bases and hence thymidine is not used. When measuring bacterial production by [^{3}H]-thymidine incorporation it is important that the *de novo* pathway is shut off, otherwise the DNA synthesis will be underestimated. Addition of large enough amounts of thymidine will inhibit the *de novo* pathway and prevent isotope dilution. A too large pool of dTTP will inhibit also the salvage pathway, so too large additions of [^{3}H]-thymidine will decrease the incorporation of thymidine. Therefore, the smallest possible concentration that gives the highest incorporation of [^{3}H]-thymidine should be used. The concentration needed to reach saturation level has to be tested experimentally, by adding different amounts of thymidine to samples incubated for a certain time. Optimally a Michaelis-Menten curve for thymidine incorporation is obtained. The right amount of thymidine to add is when there is no further incorporation of thymidine even though the dose of added thymidine increases.

Isotope dilution can occur from the *de novo* synthesis and from extracellular sources of thymidine or other compounds that compete for the same enzymes as thymidine. In water the isotope dilution is assumed to be small but in sediment the isotope dilution may be large. Thymidine easily adsorbs to particles and generally higher additions are needed to ensure that the *de novo* pathway is inhibited. To measure isotope dilution a constant amount of [^{3}H]-thymidine is added to a series of samples together with increasing amounts of unlabeled thymidine /Pollard and Moriarty, 1984/. The samples are incubated as usual and the DNA is extracted. The radioactivity is plotted against thymidine concentration and if the plotted line passes through the origo there is no isotope dilution. Isotope dilution is shown as a negative intercept. In most investigations the negative intercept has been determined from linear data. However, Robarts and Zoharty /1993/ and references therein, stress that non-linear regression is much better fit for these calculations.

During the first time period of an incubation thymidine is only incorporated into DNA. After a short time period the incorporation of thymidine stops due to degradation of thymidine by inducible thymidine phosphorylase. When the thymidine is degraded, tritiated thymidine is released and starts labelling other macromolecules than DNA. The non-specific labelling of other molecules than DNA may be extensive in natural populations and according Robarts and Zoharty /1993/ it is therefore important to extract and

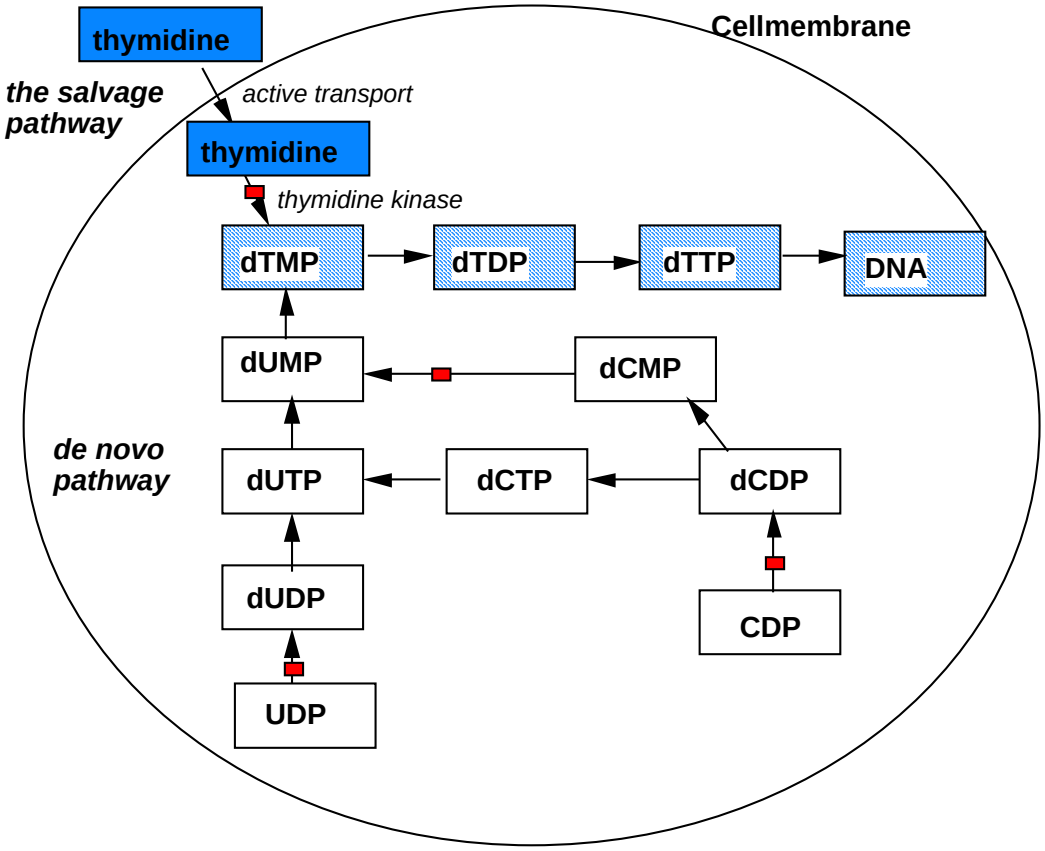

Figure 4-1. *The de novo and the salvage pathway for DNA synthesis in cells. The salvage pathway is marked with blue boxes. This pathway actively transports thymidine into the cell. Inside the cell, thymidine kinase is transforming the thymidine into dTMP (thymidine monophosphate). dTMP is also the product in the de novo pathway (clear boxes).From dTMP the transformation into dTDP to DTTP to DNA is the same for the two pathways. When measuring production via thymidine incorporation it is important that the de novo pathway is shut of. This is achieved by large enough amount of thymidine (which lead to high amount of dTTP). Red boxes on arrows indicate where feedback mechanism from dTTP occurs. Too large amounts of thymidine will also inhibit the salvage pathway, which can be seen as a red box between thymidine and dTMP.*

purify the labelled DNA. It is also important to keep the incubation time short in order to avoid incorporation into other macromolecules than DNA /Moriarty, 1990/. Bell /1993/, however, argued that there often is a perfect correlation between bacterial production and incorporation into total macromolecules if the incubation time is kept short. After 20 minutes of incubation approximately 80% of the label is in the DNA fraction but after 60 minutes less than 50% may be found in DNA. Thus, to be on the safe side it is best to extract and purify the labelled DNA. In sediment samples, DNA should always be extracted in order to avoid quenching when counting the radioactivity in a scintillation counter.

Before extraction of DNA, washing with ethanol should be performed. Radioactive label can be incorporated into lipids in the cellmembranes but this fraction is removed by ethanol washing /Wicks and Robarts, 1988/. Many extraction methods are available and a large number of them are reviewed in Robarts and Zoharty /1993/. The methods

developed by Schmidt-Tannhauser /1945/ or Schneider /1945/ have frequently been used. In the Schmidt-Tannhauser method, DNA and other macromolecules are recovered by cold acid and the DNA is separated from the RNA by alkali and precipitated by cold acid. In the Schneider method hot acid is used to extract the DNA from cells. The hot acid can result in incomplete extraction and degradation of deoxyribose and hence, the Schneider method will lead to an underestimation of the amount of DNA. The Schmidt-Tannhauser method gives much better recoveries of DNA. However, there has also been problems with the Schmidt-Tannhasuer method and DNA is not always dissolved out of the bacterial cells and has in some cases been found in the RNA fraction. Hence, modifications of the methods have been made. One adaptation, usually referred to as the acid-base hydrolysis method, is to do the alkali step first and then add acid. This will increase the amount of extracted DNA. Another method is to drop the alkali step, and use cold acid, assuming that the DNA will compromise a fixed fraction of the macromolecules. This method is referred to as the cold TCA precipitation method /Robarts and Zoharty, 1993/.

After the extraction the radioactivity is measured and the incorporation of thymidine can be calculated. A conversion factor is used to calculate the production in $gC \cdot l^{-1} \cdot hour^{-1}$.

$$\text{Moles thymidine} \cdot \ l^{-1}\ h^{-1} = ((dpm_{sample}\text{-}dpm_{blank}) \cdot 1.665 \cdot 10^{-2} \cdot 10^{-3} \cdot a) / (SA \cdot t \cdot V)$$

where

$1.665 \cdot 10^{-2}$	number of Bq per dpm
10^{-3}	mmol/mol
a =	conversion factor for volume of formaldehyde/ethanol added a = 1.03 if assuming 1% of final concentration
SA =	the specific activity of the ^{3}H-thymdine solution in Bq per mmol
t =	incubation time in hours
V =	filtered volume in litres.

$$P = \text{moles thymidine} \cdot l^{-1} \cdot h^{-1} \cdot \ (\text{cells / mole}) \cdot \ (\text{Carbon / cell})$$

where

P =	heterotrophic bacterial production in $g\ C \cdot l^{-1} \cdot h^{-1}$.

The conversion factors for determining how much carbon is produced per amount of incorporated thymidine is either gained by experiments or by theoretical factors. Bell /1990/ came to the conclusion that conversion factors between 1 and $2 \cdot 10^{18}$ cells per mole incorporated thymidine are the most realistic.

The thymidine method is widely used and well developed. However, it is uncertain whether the thymidine can be incorporated into all bacteria. Anaerobic and chemolitotrophic bacteria have been shown unable to incorporate thymidine or to incorporate thymidine to a lesser extent than aerobic bacteria /Pedros-Alió and Newell, 1989; Johnstone and Jones, 1989; Gilmour et al., 1990/. However, other studies show that anaerobic and chemolitotrophic bacteria are able to incorporate thymidine /Kraffzik and Conrad, 1991/. McDonough et al. /1986/ showed that thymidine was incorporated in bacteria even during low oxygen concentrations but gave smaller production values than the leucine method. However, it is clear that caution has to be taken when it comes to applying the thymidine method in anaerobic samples.

4.1.1 Thymidine incorporation in water

It is quite easy to measure thymidine incorporation in water and one person can do 50 samples on one day /Bell, 1993/. In water ≈ 20 nM [^{3}H]-thymidine is a recommended concentration /Moriarty, 1990/. In environments with high particulate matter a higher concentration may be needed so a test of sufficient thymidine concentration should always be made. A practical way to measure thymidine incorporation in water is to use glass scintillation vials that can be hung on the actual depth in the lake during incubation.

4.1.2 Thymidine incorporation in sediment

It is more difficult to measure thymidine incorporation in sediments than in water. Experiments have to be performed to find the right amount of [^{3}H]-thymidine to add. [^{3}H]-thymidine adsorbs to particles and there is a larger risk for isotope dilution in sediments than in water. Therefore, it is important to perform an isotope dilution experiment. The isotope dilution can also vary with the amount of sediment used. Moriarty and Pollard /1990/ found that in 0.8 g sediment isotope dilution occurred, but in 0.2g sediment no isotope dilution occurred.

Another difficulty regarding sediment measurements is that often not all bacteria are exposed to the same amount of thymidine. Distribution of thymidine evenly into sediment during incubation will disturb the sediment. Findlay et al. /1985/ suggests injecting the [^{3}H]-thymidine into sediment cores. However, some bacteria may still not be exposed to high enough concentrations if they are too far away from the source. A common way to ensure that all bacteria are exposed to high concentrations is to make a slurry of sediment and thymidine. This of course disturbs the initial composition of the sediment. Because of the disturbance there may be a change in the activity within the bacterial community as environmental conditions like redox potential, pH etc are altered. However, there is a lag period between a disturbance and the change in DNA synthesis. At least surface sediment can be disturbed without differences in thymidine incorporation if the incubation time is short /Moriarty, 1990/. Deep sediments, with many inactive bacteria, may need a long incubation time for the thymidine incorporation to be detectable. This means that the incubation time may be long enough to record effects of a disturbance on DNA synthesis when resting cells start to grow.

4.1.3 Thymidine incorporation in epiphytic bacteria

Very high concentrations of thymidine are needed to reach saturation levels for epiphytic bacteria, 400 nM [^{3}H]-thymidine has been reported /Theil-Nielsen, 1999/.

Bacteria on macrophytes may be scraped or shaken off the macrophyte but more commonly they are incubated in a bottle together with a piece of the macrophyte. It is preferable if the bacterial production can be measured on intact macrophytes because then the interaction between macrophytes and bacteria is not affected.

4.2 Leucine incorporation

Leucine is a small amino acid, which is easily incorporated into the protein of bacteria. Bacteria contain approximately 60% protein /Kirchman, 1993/ and the incorporation of leucine provides a direct measurement of the bacterial carbon production. Ten time more leucine than thymidine is incorporated into bacterial cells when synthesising an equivalent amount of biomass and hence, the leucine method is an order of magnitude more sensitive than the thymidine method. The molar fraction of leucine is relatively constant in proteins so an increase in leucine incorporation reflects an increase in bacterial biomass and not a shift in the molar fraction of leucine /Kirchman et al., 1985/. 50% of the bacterial assemblage took up leucine and >90% of it was incorporated into protein in a study by Kirchman et al. /1985/. Leucine is incorporated into bacteria even during low oxygen concentrations /McDonough et al., 1986/ so leucine may be a good complement during anaerobic conditions. Leucine incorporation has been shown to be constant for up to 36 hours so the leucine method does not seem to be dependent on incubation time /Marxsen, 1996/.

As for the thymidine method, leucine is added to samples and incubated *in situ* at the preferred depth. Leucine can be incorporated both as [^{3}H]-leucine and as [^{14}C]-leucine. The incubations may be stopped with trichloroacetic acid (TCA) and, after washing with TCA and ethanol the radioactivity can be measured.

As for thymidine, the appropriate addition of leucine has to be experimentally tested in order to avoid the *de novo* synthesis and isotope dilution. To inhibit the internal production, high enough doses of leucine has to be added. Addition of extracellular leucine has been shown to inhibit the internal leucine production in natural marine bacterial assemblages /Kirchman et al., 1985/. The bacteria could theoretically use the added leucine as a carbon source, but in the amounts usually used this is not a problem. Fisher and Push /1999/ showed that bacterial activity was not enhanced at micromolar concentrations of leucine. Too high doses of leucine may also make the leucine available to other microorganism e.g. algae. Isotope dilution has to be measured in the same way as for thymidine incorporation.

In addition to being incorporated into proteins, leucine may be adsorbed to the lipids on the cell membranes. This leucine has to be washed away with ethanol before measuring the radioactivity /Wicks and Robarts, 1988/. Extraction of protein may not be necessary since most of the leucine is incorporated into protein and protein makes up the largest part of the bacterial cell /Kirchman, 1993/. One way to measure the incorporated leucine is by combustion. Tuominen /1995/ showed that a combustion method with

prior ethanol washing yielded more consistent results than the extraction methods with TCA. When compared to samples where the particles were bound in a gel the gel samples yielded on average 81% of the combusted samples. Where the particles where settled on the bottom of scintillation vials the yield was on average 37% of the combusted samples. However, the combustion technique has also been claimed to overestimate the heterotrophic bacterial production /Fisher and Push, 1999/. Hietanen et al. /1999/, found a good correlation between the gel method and the combustion method. Recovery of added leucine should always be tested since different turbidity of the water and different methods yield different recoveries /Amon and Benner, 1998/. Production is calculated in g $C \cdot l^{-1} h^{-1}$ by the following calculation:

$$\text{Production} = \text{Leu} \cdot 131.2 \cdot (\%\text{leu})^{-1} \cdot (\text{C}/\text{Protein}) \cdot \text{ID}$$

where:

Leu =	rate of leucine incorporation
131.2 =	molecule mass of leucine
%leu =	molar fraction of leucine in protein
C/Protein =	ratio of cellular carbon to protein
ID =	isotope dilution.

Problems with the leucine method are 1) protein turnover and 2) incorporation by other organisms than bacteria. Microbial cells can synthesise and degrade protein independent of net growth, which is termed protein turnover /Kirchman, 1993/. If the growth rates are low the protein turnover can lead to serious overestimates of the bacterial growth. Leucine may also be incorporated into fungi /Fallon and Newell, 1986/. However, during short incubations (less than 30 minutes), the bacteria should be the main users because of their faster uptake of small organic molecules /Tuominen, 1995/. The low concentrations of leucine used during the incubation is probably too low for eukaryotic organisms to utilise since eukaryotes have been shown to incorporate leucine first at concentrations above 200 µM /Fisher and Push, 1999/.

4.2.1 Leucine incorporation in water

Samples can be incubated at preferred depths. In eutrophic environments it is possible to dilute the leucine with nonradioactive isotope. For example can 0.5–1 nM radioactive isotope be used together with 9–9.5 nM non-radioactive isotope. This will decrease the cost of the experiment. In highly oligotrophic environments only radioactive leucine should be used.

4.2.2 Leucine incorporation in sediments

Incubation with leucine in sediments may be done both as slurry of the sediment and as intact sediment cores. The methods yield similar results /Fisher and Pusch, 1999; Marxsen, 1996/. Compared to thymidine incorporation, smaller amounts of sediments may be used, and the leucine method becomes cheaper than the thymidine method. However, when the sediment material is coarse and heterogeneous it can be difficult to take small samples that are representative for the sediment.

4.2.3 Leucine incorporation in epiphytes

Leucine incorporation can be measured on intact macrophyte tissues. However, very high leucine concentrations are needed to reach saturation level and values of 300–1100nM have been reported /Thomas and Wetzel, 1995; Theil-Nielsen, 1999; Törnblom and Søndergaard, 1999/. This makes the method very expensive.

4.3 Frequency of Dividing Cells (FDC)

Frequency of dividing cells (FDC) is determined in microscope. The frequency of dividing cells in the sample is assumed to reflect the average growth rate /Hagström et al., 1979/. FDC is not a commonly used method but has the advantage that it does not need incubation with radioactive tracers. A problem with the method is that it can be difficult to determine dividing cells in an epifluorescence microscope. In a transmission electron microscope (TEM), however, it is easy to determine the frequency of dividing cells. Tuiomi /1997/ compared the FDC counted in an epifluorescence microscope and a TEM. She found a good correlation for larger bacteria while smaller ones were difficult to detect in the epifluorescence microscope.

The FDC method has not been used in a wider extent, mainly because there is no good conversion factor from FDC to μ (the average growth rate).

FDC often overestimates the bacterial production compared to the thymidine and leucine methods /Riemann and Bell, 1990; Fallon and Boylen, 1990/. Fallon and Boylen /1990/ found higher production values when using the FDC method compared to the thymidine incorporation method. They also measured the respiration and found that the thymidine values corresponded well with what was predicted from the respiration whereas the FDC values were too high.

5 Primary production

Primary production is here defined as the production of organic compounds from CO_2 and nutrients during light.

$$6\ CO_2 + H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$$

The primary production has often been investigated in the water column of lakes, but the benthic as well as the epiphytic primary production have been studied to a lesser extent. In marine coastal areas the benthic production has been investigated more thoroughly than in lakes and it has been shown that the benthic primary production may exceed the integrated primary production in the water column /Cahoon and Cooke, 1992; Barranguet et al., 1996; Carmouze et al., 1998/. Also epiphytic primary production can contribute significantly to the primary production of lakes /Burkholder and Wetzel, 1989/. Primary production is most commonly measured by incorporation of $^{14}CO_2$ or by oxygen production in dark and light bottles. The change in dissolved inorganic carbon (DIC) is a similar method to the oxygen production in dark and light bottles but has been used to a lesser extent. Two promising, rather new methods, one of the methods using oxygen microelectrodes and the other measuring photosynthetic parameters, will also be discussed in this report.

5.1 $^{14}CO_3$-fixation

Primary production is commonly measured by the fixation of radioactive CO_2. The method was developed for water primary production by Steeman Nielsen /1952/ and has been further developed since then. Samples are incubated with radioactive ^{14}C and during photosynthesis algae and cyanobacteria incorporate the tracer. The $^{14}CO_3$-fixation method is a sensitive method and has a detection limit of 0.01 $mgC \cdot m^{-3} \cdot d^{-1}$. It has been shown that ^{14}C uptake is a good estimate of growth even during short incubations /Ahlgren, 1991/.

When samples are taken they should be kept dark until the incubation. Care should be taken to avoid exposing deep samples to sunlight since the high irradiance of direct sunlight may inhibit the photosynthesis of algae and cyanobacteria. The ^{14}C is commonly added as $NaH^{14}CO_3$. Enough ^{14}C should be added to so the fixed radioactive label can be measured but care has to be taken not to disturb the CO_2 equilibrium in the water. Incubations are commonly made during midday for a couple of hours. The incubation should be limited to four hours to minimise “bottle effects”. Nutrients may become limiting if the experiment is run for too long time, since natural mixing and exchange of water is prevented in the bottles. Sampling should always be made at the same time of the day. Termination of the incubations can be made with e.g. formaldehyde.

Both light and dark incubations have to be made. Bacteria and non-biological processes may also fixate ^{14}C and the dark fixation gives a measure of these processes, which must be subtracted from the results of the light incubations.

When the total content of CO_2 and the amount of added ^{14}C is known, the rate of primary production P_t can be measured. The P_t can be used together with the irradiance measurements to calculate the primary production for the whole day. The irradiance should be measured for at least half a day, e.g. from sunrise to true noon or from true noon to sunset.

$$P_t \ (mgC \cdot m^{-3} \cdot h^{-1}) = (dpm_1 \cdot \text{total } CO_2 \cdot 12 \cdot 1.05 \cdot k_1 \cdot k_2 \cdot k_3) / (dpm_2)$$

where:

$P_t \ (mg\ C \cdot m^{-3} \cdot h^{-1})$	production per time and m^3
dpm_1	$sample_{light}$ dpm – $background_{dark}$ dpm= net dpm/sample
Total CO_2	concentration of total CO_2 in experimental water mM /dm^3
12	the atomic weight of carbon
1.05	a correction factor for ^{14}C discrimination. The C^{14} is taken up 5% slower than the ^{12}C
K_1 =	a correction for subsampling. For example of 15 cm^3 were used from a total sample of 27 cm^3 and 1 cm^3 ^{14}C were added then k_1= 28/15
K_2 =	time correction factor. If the incubation is 2h k_2 is 0.5
K_3 =	a unit conversion factor. When converting mg C $\cdot$ dm^{-3} to mgC $\cdot$ m^{-3} K_3 is 10^3
dpm_2=	activity of the added ^{14}C solution in dpm.

An advantage with the ^{14}C-incorporation method is that it can be used also during anaerobic conditions /Revsbesh and Jørgensen, 1983/. Another advantage is that the production is measured as carbon, which is the common way to express primary production.

A disadvantage with the method is that the incorporation gives a measure of something between the net and gross production. Gross primary production is the primary production including respiration and excretion. Net primary production is the gross primary production minus respiration and excretion. Bender et al. /1987/ found that the ^{14}C-method for a planktonic community gave production values of 60 to 100% of the gross production measured by a CO_2 light dark method which indicates that the ^{14}C production method is not fixed in proportion to other primary production community measurements. Short incubation times gives values that approach gross production whereas longer incubation times approach net production /Ahlgren, 1970/. Another disadvantage

with the method is that it is time consuming and rather difficult and where different laboratories compared their ^{14}C fixation methods the results varied with up to 15% /Richardson, 1991/.

5.1.1 ^{14}C-incorporation in water

It is easier to measure the ^{14}C incorporation in water samples than in sediment or on macrophytes. Water samples can be incubated in bottles at the actual depth. To be able to calculate the primary production for the whole water column, measurements have to be made at different depths to cover the whole photosynthesis depth profile.

Plexiglass or high quality glass, e.g. Pyrex, should be used since normal silica glass absorbs short wave length radiation /Findenegg, 1966/. Sample bottles have to be carefully cleaned e.g. with HCl, to avoid bacterial biofilm and toxic substances on the inside.

Water samples can be filtered to obtain the incorporated ^{14}C on the filter. Another way to treat the water before the scintillation counting is to add hydrochloric acid and bubble with air to remove the unincorporated ^{14}C /Schindler et al., 1972/. This will remove dissolved inorganic carbon but dissolved organic carbon and particulate organic carbon will remain in the sample.

5.1.2 ^{14}C-incubations in sediment

It is more difficult to measure primary production with the ^{14}C incorporation method in the sediments than in the water. Sediment samples should be incubated at the surface of the sediment. Caution has to be made so the samples remain at the surface and do not sink into the sediment.

The respiration is higher in sediments than in the water. Some incubations with ^{14}C in sediment have been done as sediment slurries /Gargas, 1970; Schreiber and Pennock, 1995/. Others have been made with intact sediment cores where additions of the labelled ^{14}C has been made either to the overlaying water /Van Raalte et al., 1974; Cadée and Hegeman, 1974/ or incorporated into the core /Jönsson, 1991/. In the percolated core, labelled carbon is added to the overlaying water and allowed to penetrate down through the photosynthetic active layer. In this way all the algae and cyanobacteria is exposed to the same concentration of the radioactive label. With the slurry technique, algae and cyanobacteria from deeper layers get exposed to higher light intensities than they are in the natural undisturbed sediment and the primary production is overestimated /Jönsson, 1991/. When the ^{14}C is added to the overlaying water of an intact core, it may not reach all the photosynthetic algae and the primary production may instead be underestimated /Jönsson, 1991/. If ^{14}C is added to the overlaying water a stirring mechanism should be used. This increases the diffusion of the ^{14}C into the sediment. As an example, the primary production of benthic diatoms was increased twofold when stirring the overlaying water in a study by Gould and Gallagher /1990/.

5.1.3 ^{14}C-incorporation in epiphytes

In early studies of the epiphytic primary production epiphytes were scraped from the macrophytes and then incubated to determine the primary production. In later investigations this has not been done sine the effect of removing the epiphytes from the macrophytes is not known. The productivity of the epiphytes can be measured in intact

bottles using the ^{14}C-method (e.g. Cattaneo and Kalff, 1979/. After the incubation the epiphytes can be removed by e.g. scraping or shaking the leaves and the radioactivity can be measured.

5.2 Oxygen production

The oxygen production method was described by Garder and Gran /1927/ and is an inexpensive and simple method. During photosynthesis, O_2 is formed and by measuring the concentration of dissolved oxygen (DO) before and after incubation in light and dark bottles the primary production can be calculated. Dark incubations are used to compensate for respiration and are subtracted from the light incubations when calculating the primary production. The DO can be measured either by an oxygen electrode or by Winkler titration.

To assess the primary production in carbon units a photosynthetic quotient (PQ) has to be calculated. The PQ is a value of how much carbon is produced per molecules of liberated oxygen. The PQ is dependent on what nitrogen sources that are available and what chemical substances are produced /Williams et al., 1979/. In an analysis of 200 paired measurements of DO and ^{14}C measurements the PQ values varied from 0.5 to 3.5 /Williams and Robertson, 1991/. Hence, in order to calculate carbon production, the photosynthetic quotient should be estimated separately. The net photosynthesis is calculated with the following formula:

$$\text{Net photosynthesis} = \frac{[(O_2L)-(O_2D)] \cdot (1000) \cdot (0{,}375)}{(PQ \cdot t)}$$

where:

Gross photosynthesis = production in $mgC \cdot m^{-3} \cdot h^{-1}$

O_2L = oxygen in light incubations

O_2D = oxygen concentration in dark incubations

0.375 = to convert oxygen to carbon, moles carbon to moles oxygen ($12\ mgC/32mgO_2 = 0.375$)

PQ = photosynthetic quotient

t = time in hours.

The oxygen production method has earlier been restricted to productive waters since it has not been sensitive enough to use in oligotrophic waters. However, Carignan et al., /1998/ has shown that with some modification, the oxygen production method in light and dark bottles can be used even in oligotrophic waters with a detection limit of $2\ \mu g \cdot l^{-1}$.

The largest objection to the oxygen method is that oxygen respiration may not be the same in light as in dark bottles. Cohen et al. /1977/ indicated that the oxygen consumption might be higher in light than in dark, which would lead to underestimation of the primary production. Sometimes when the oxygen production method has been used,

unexpected results as oxygen production in dark bottles has been found /Pamatmat, 1997/. Pamatmat /1997/ explains this with H_2O_2 processes and claim that the oxygen production method is unattainable.

Revsbesh and Jørgensen /1981/ showed that in highly oxygenated sediments there were almost identical results with the oxygen method, the $^{14}CO_3$-fixation method and the oxygen microprofile method at low light intensities. At higher light intensities, however, the oxygen method grossly underestimated the primary production due to bubble formation.

The oxygen method can not be used during anaerobic conditions.

5.2.1 O_2-production in water samples

Water samples can be incubated at the actual depth and dark incubations should be included. As for the ^{14}C incorporation method samples should be incubated in high quality glass bottles or plexiglass bottles at different depths, which makes it possible to calculate the production for the integrated water column.

5.2.2 O_2-production in sediment samples

On the bottom, enclosures can be pressed into the sediment. It is important that the incubation chambers used are not made of stainless steel, since the steel works as cathodes and reduces the oxygen in the chambers /Cramer, 1989/. A sample of the overlaying water is withdrawn at the start and at the end of the incubation.

Animal burrows in the sediment that are trashed when putting down the incubation chambers may severely influence the DO. Different amounts of animals trapped in the different incubation chambers will differ the respiration and hence the DO. If the animals are counted after the incubation it is possible to compensate for the animal respiration.

5.2.3 Oxygen production of epiphytes

The oxygen method can not be used when determining the primary production of intact epiphytes since there is no distinction between the plant production and the epiphytic production. By removing the epiphytes from the macrophytes prior to incubation the oxygen production method can be used. However, this will influence the natural condition and hence, the measurement will not reflect the true primary production of the epiphytes.

Instead, artificial substrates can be used when measuring the production of epiphytes via oxygen production. In this case, the composition and amount of epiphytes may not be the same as on the natural substrate. Neither may the conditions be the same since many studies indicate that the epiphytes gain nutrients from the substrate where they are attached /Harlin, 1973; McRoy and Goering,.1974; Moeller et al., 1988/. Hence, the epiphytic primary production may preferably be measured by ^{14}C-additions. However, some studies have shown similar results when plastic vs natural plants have been used /Cattaneo and Kalff, 1979/.

5.3 O_2-microprofile method

Primary production can be measured by O_2-microelectrodes. In benthic studies, microelectrodes are lowered into the sediments. The oxygen microprofile method measures the rate of oxygen depletion after a shift from light to dark conditions. At steady state, which is achieved after a certain time, the photosynthetic oxygen production in a layer will balance the loss due to respiration and diffusion of oxygen away from the layer. When the light is stopped diffusion and respiration are initially the same and therefore the rate of oxygen depletion in the first seconds will equal the former production. /Revsbech and Jørgensen, 1983/.

The method requires a microelectrode with a tip of 2–10 µm and hence gives a very good vertical distribution of the production /Revsbech and Jörgensen, 1983/. The spatial resolution is ca 0.1 mm and the O_2 concentration is determined within 1s. A problem with the method is that the porosity has to be measured and it can be difficult to separate the sediment in small vertical layers. Revsbesh and Jørgensen /1981/ could not separate the 0–1.5 mm layer of the sediment and hence used the 0–2.5 mm layer, which lead to an underestimation of the productivity.

Gas bubble formation has been put forward as a problem with oxygen methods but because of the good spatial resolution this is negligible with the oxygen microelectrode method. It is possible to measure the amount of gas bubbles within the enclosure chambers and they can also be calculated from saturation.

5.4 Changes in dissolved inorganic carbon (DIC)

Changes in dissolve inorganic carbon (DIC) can be used to calculate the primary production. CO_2 is used in the photosynthesis and hence, the concentration of DIC should decrease during primary production. Light and dark bottles are incubated as for the oxygen production method and the DIC concentrations are measured before and after the incubations. The dark bottles represent non-photosynthetic uptake of DIC and should be subtracted from the light bottles in the calculations of primary production. The DIC concentrations can be measured directly with modern equipment but can also be determined by alkalinity. CO_2 is calculated from alkalinity and pH with the following formula:

$$\text{Total } CO_2 = \text{Carb-Alkalinity} \cdot (1+a_H/K_1' + K_2/a_H)/(1+2K_2/a_H)$$

where:

Carb-alkalinity = Alkalinity-($OH^- + H^+$) (The substarction is only necessary when pH is >8.8.)

$K_1' = (H^+ \cdot HCO_3^-) / (CO_2 + H_2CO_3)$

$K_2 = (H^+ \cdot CO_3^{2-}) / (HCO_3^-)$

Numerical values can be used for K_1' and K_2 and then total CO_2 can be calculated as:

Total CO_2= Carb-Alkalinity $\cdot f_{pH,T}$

where

$f_{pH,T}$= a correction factor to obtain CO_2 from titration alkalinity, pH and temperature. A graphical representation of the factors $f_{pH,T}$ calculated for ionic strength = 0 (sufficient approximation for most freshwaters), are given in Vollenweider /1971/.

An advantage with the method is that the production is expressed in gC which is the common way to express primary production. The changes of carbonate concentrations may be small and the DIC method is not as sensitive as the ^{14}C method /Strickland and Parson, 1968/.

There may be some changes in DIC concentrations during the transport from the lakes to the laboratory if the transport is long. However, one can assume that the change is the same for both light and dark bottles /Ahlgren personal communication/.

5.5 Photosynthetic parameters

Primary production measured by fluorescence overcomes the problems with light and dark bottles since it measures the productivity at instantaneous measurements. There is neither an effect of the bottle since no bottle is used. The measurements is rapid, convenient and continuous, and without bottle effect. The problem with the method is that the measurements have to be calibrated often and is only stable for a restricted time when the algal community is relatively constant /Öquist et al., 1982/. However, the method has been existing for a while, it hasn't been used in a wider extent *in situ* and more work has to be done before it can be used in a larger scale. However, it is a promising method for the future since it is cheap and fast.

According to the theory, the chl *a* fluorescence yield is proportional to the electron flow through photosystem II and hence to the primary production. Fluorescence signal can be measured with a pump and probe fluorometer /Kolber and Falowski, 1993/ and, together with irradiance measurements, the photosynthesis over time can be calculated. The method requires conversion factors for determination of production in carbon and more work has to be done to obtain reliable conversion factors.

This is a relatively new technique and until recently it has not been tested *in situ*. Gorbunov et al. /2000/, however, has tested a scuba fluorometer, which can be carried by a diver over the sediments. This has the advantage of not disturbing the sediment.

6 Results from illuminated lake sediments

Microbial studies on illuminated lake sediment habitats have to a large extent focused on heterotrophic bacterial biomass and production. Fewer studies have been made of biomass and production of primary producers. Most studies of benthic algae and cyanobacteria and benthic primary production have been performed in coastal marine areas but more and more studies are now performed also in lakes.

Bacterial biomass is high in lake sediments and usually varies between 10^8 and 10^{10} cells per cm^3 /Schallenberg et al., 1989/. The number of bacteria in sediment does not vary much over the year or between years /Boström et al., 1989/. Also the bacterial production may be high in lake sediments. However, there is usually no correlation between bacterial biomass and bacterial production.

Fewer studies have been made of biomass of microphytobenthos and primary production on lake sediment habitats. However, the few studies that have been performed indicate that, in lakes where a large proportion of the bottom area receives enough light to support photosynthesis, benthic algae can dominate carbon fixation /Hargrave, 1969; Björk-Ramberg, 1981; Wetzel, 1996/. Also in lakes where the light exposed littoral zone is narrow the benthic algae can make a large contribution to the total primary production in a lake /Loeb and Reuter, 1981; Heath, 1988/. Heath /1988/ found that in Watts Lake, in depths between 4 and 35m, the annual benthic primary production was 5.5 $gC \cdot m^{-2}$ whereas the phytoplankton annual production was 10.1 $gC \cdot m^{-2}$.

The biomass of benthic algae and cyanobacteria can be of much higher magnitudes than the biomass of planktonic algae and cyanobacteria. In a study in a subarctic lake in Sweden the biomass of the benthic algae was 20 times higher than the biomass of the phytoplankton in the overlaying water column /Björk-Ramberg, 1981/. In some lakes the benthic algae are not gathered at the shallowest part of the lake but e.g. in Lake Michigan the diatom abundance was greatest at 9 and 15m compared to 7, 23, and 27m /Stevensen and Stoermer, 1981/. The algal communities at 23 and 27 meters depth were mainly made up by fallout from the planktonic community. Other studies, however, have reported that the deep benthic communities have been truly benthic and very taxonomically diverse /Caljon and Cocquyt, 1992; Kingston et al., 1983/. Biovolumes of benthic algae generally decrease with depth but some taxa e.g. *Pinnularia* has shown weak positive correlation with depth indicating that they are adapted to low light conditions and may be facultatively heterotrophic /Stevenson et al., 1985/.

In Lake Bobo in West Africa the biomass of benthic algae was found to be higher than the phytoplankton biomass during most part of the year /Havens et al., 2000/. Only when the macrophytes died and released nutrients to the water the phytoplankton biomass exceeded the benthic algal biomass. The theory is that benthic algae and macrophytes can outcompete the phytoplankton the rest of the year since they can take up nutrients directly from the sediments. Riber and Wetzel /1987/ and Carlton and Wetzel /1988/ demonstrated that epipelic periphyton communities play a significant role in releasing phosphorous from the sediment and thereby generate supplies for their own phosphorous demand.

It seems like the primary as well as the heterotrophic production on illuminated lake sediments can have a large impact on the total production in lakes. However, there is still need of further research to be able to determine the role of benthic algae and bacteria in lakes. Although some studies indicate that the benthic algae can make up a major part of the biomass and primary production of a lake the sinks and pathways for the fixed carbon are poorly known. Moreover, the studies that have been performed have been conducted in the upper littoral zone, while less is known about the deeper infralittoral zone. In the infralittoral zone the maximum taxonomic periphyton diversity is found, but the quantitative role of the community and why it is so diverse is yet to be elucidated /Lowe, 1996/. In conclusion, the benthic primary production and heterotrophic bacterial production can make a large contribution to total lake turnover and hence it is important to include also the benthic community when studying lake ecosystems.

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