Biodegradation **9:** 393–409, 1998.

Nuclear magnetic resonance in environmental engineering: Principles and applications

P.N.L. Lens & M.A. Hemminga
Laboratory of Molecular Physics, Wageningen Agricultural University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands

Key words: biodegradation, bioreactors, NMR imaging, NMR spectroscopy, nutrient removal, sludge, solid waste

Abstract

This paper gives an introduction to nuclear magnetic resonance spectroscopy (NMR) and magnetic resonance imaging (MRI) in relation to applications in the field of environmental science and engineering. The underlying principles of high resolution solution and solid state NMR, relaxation time measurements and imaging are presented. Then, the use of NMR is illustrated and reviewed in studies of biodegradation and biotransformation of soluble and solid organic matter, removal of nutrients and xenobiotics, fate of heavy metal ions, and transport processes in bioreactor systems.

Abbreviations: CP – cross-polarization; ESR – electron spin resonance; GC – gas chromatography; GPS – glycerol 3-phosphorylserine; HPLC – high pressure liquid chromatography; LC – liquid chromatography; MAS – magic-angle-spinning; MRI – magnetic resonance imaging; NMR – nuclear magnetic resonance; PFG – pulsed field gradient; PHB – polyhydroxybutyrate; PHV – polyhydroxyvalerate; P_i – inorganic phosphate; ppm – parts per million; SOM – soil organic matter; T_1 – spin-lattice relaxation time; T_2 – spin-spin relaxation time; TMS – tetramethylsilane.

Introduction

Nuclear magnetic resonance (NMR) is a spectroscopic technique based on the magnetic properties of atomic nuclei. As NMR spectroscopy reveals useful information on molecular structure and chemical reactions, it has rapidly progressed to become a powerful non-destructive analytical tool. The development of multi-dimensional NMR techniques has resulted in a breakthrough in the determination of the three-dimensional structure of proteins, nucleic acids, and carbohydrates. In addition, by using magnetic resonance imaging (MRI), it is possible to produce an image of the water distribution in a sample. For this technique linear magnetic field gradients are generated that enable to discriminate between different spatial locations in the object.

Apart from these well-known application fields, NMR analyses can also be applied to study a whole range of processes relevant to environmental engineering. The NMR techniques currently available offer the possibility to study, *in situ*, simultaneously (bio)degradation and transport processes. This paper aims to review applications of NMR that have been used in environmental science and engineering. Firstly, the concepts and terminology commonly used by NMR specialists and widely encountered in the NMR literature are introduced. The use of NMR techniques to study environmental engineering applications will be examined next. Developments in NMR spectroscopy and imaging of special interest to environmental engineers will be the final topic.

NMR measurements: basic principles

Magnetic resonance

A full grasp of NMR fundamentals is not essential to evaluate potential applications of NMR spectroscopy, but evaluation of suitable NMR experiments does require some basic knowledge of the basics of NMR. Therefore, in this paper the most essential concepts are presented. The reader is referred to other recent review papers for more details about the fundamentals of NMR measurements (Veeman 1997; Hemminga 1992).

The proton/neutron ratio of certain atomic nuclei (e.g., ^{1}H, ^{13}C, ^{31}P) results in a magnetic property that is called spin (Table 1). The spinning motion produces a small magnetic moment μ, that in turn produces a dipolar magnetic field. Therefore these nuclei will have an interaction with an externally applied steady magnetic field B_0, but also they will exhibit mutual magnetic interactions. Dictated by quantum mechanical rules, these interactions lead to different energy states of the spins. By applying electromagnetic radiation of a frequency that corresponds to the energy differences, these energy states can be examined similar as in optical spectroscopy. This is called: nuclear magnetic resonance (NMR) spectroscopy. Nuclei that have no spin, do not have a magnetic moment and experience no magnetic interactions. Therefore such nuclei do not exhibit magnetic resonance effects. This applies, for example, to the common carbon (^{12}C) and oxygen (^{16}O) nuclei. Useful candidates for nuclear magnetic resonance experiments are protons (^{1}H) and a stable carbon isotope (^{13}C), which are present in nature for 100 and 1.11%, respectively. Only NMR active isotopes are listed in Table 1.

At a magnetic field in the range of Tesla's (T) that is commonly used in NMR spectroscopy, the electromagnetic frequencies are in the MHz-range, the radio-frequency (rf) range, which are generated by radio transmitters. Modern NMR equipment irradiates samples with bursts (pulses). The subsequent dynamics of the spins are monitored and converted into spin echo decay curves (relaxation measurements) or into spectra (spectroscopy). In general, there is only a slight excess of nuclear spins in the lowest energy level (about 5 on 100,000 protons), because the energy differences are so small. Consequently NMR spectroscopy is an insensitive technique and in general an advanced NMR detection system is necessary to achieve a satisfactory signal-to-noise ratio and an acceptable measuring time.

Relaxation times

The relaxation of the spins is governed by two time constants, designated by T_1 and T_2. The spin-lattice relaxation (T_1) describes the exchange of spin energy with the environment, whereas the spin-spin relaxation time (T_2) is related to the width of a resonance line in an NMR spectrum, in that the line width is inversely proportional to T_2. Both relaxation times are affected by the molecular mobilities in a system, and the presence of spins from paramagnetic ions. These two types of relaxation can be quantified using specific pulse sequences. For a review of this type of measurements, see Kenyon (1992).

Chemical shift

The basis of the application of NMR spectroscopy in chemistry is the chemical shift. Atomic nuclei are surrounded by an electron cloud, whose structure depends on the atom involved and its position in the molecule. If the atom is subjected to an externally applied magnetic field, a rotational movement is induced in the electron cloud, which induces a small magnetic field at the site of the nucleus that tends to counteract the external magnetic field. Consequently, the nucleus experiences a slightly reduced magnetic field, so that it will resonate at a slightly different frequency. For a given external magnetic field, nuclei in different chemical environments resonate at different frequencies. This phenomenon is called chemical shift. From the chemical shift, the NMR spectroscopist can deduce in which chemical group a particular nucleus is present. The chemical shift is proportional to the frequency of the applied rf radiation and is therefore expressed as parts per million (ppm).

For example, a value of 1 ppm implies that two NMR resonances are separated by 100 Hz in a spectrometer operating at 100 MHz. By convention a relative scale is adopted that refers the chemical shift to a proton in tetramethylsilane (TMS, $(CH_3)_4Si$), as given by:

$$\text{Chemical shift in ppm} = \frac{\text{Chemical shift from TMS in Hz}}{\text{Spectrometer frequency in Hz}} \times 10^6$$

The chemical shift increases at higher magnetic fields, and concomitantly the resolution of the spec-

Table 1. Selected NMR nuclei with their basic properties

Isotope	Spin	NMR frequency (MHz) at 4.7T*	Relative sensitivity	Natural abundance (%)
^{1}H	1/2	200.00	100.00	99.98
^{13}C	1/2	50.29	1.59	1.11
^{15}N	1/2	20.27	0.10	0.37
^{17}O	5/2	27.11	2.91	0.04
^{19}F	1/2	188.15	83.00	100.00
^{23}Na	3/2	52.90	9.25	100.00
^{27}Al	5/2	52.11	21.00	100.00
^{29}Si	1/2	39.73	0.78	4.7
^{31}P	1/2	80.96	6.63	100.00
^{39}K	3/2	9.33	0.05	93.10

* The SI unit of magnetic field strength is tesla (T). It is equivalent to 10000 Gauss in cgs units.

trometer increases, because the resonance lines become better separated. In addition the sensitivity increases as well. For that reason, there is a strong tendency to employ NMR spectrometers at higher magnetic fields, especially for (bio)molecules that contain a large number of nuclei. Figure 1 illustrates the chemical shifts in a ^{31}P NMR spectrum, resulting from the differences in the position of the ^{31}P nucleus in the molecules.

Dipole-dipole interaction

When nuclei come close together, their magnetic moments will experience a magnetic interaction. This effect is called dipole-dipole interaction, which is active through space. Dipole-dipole interactions give rise to very broad NMR spectra in solids systems (Preston 1996). Therefore, NMR spectroscopy on solids requires a special approach, as discussed below. In liquids, however, the dipole-dipole interactions are still there, but the fast tumbling motions of the molecules result in an averaging of the interactions. In this situation, the dipole-dipole interactions still affect the nuclear relaxation that gives rise to the widths of the NMR lines (T_2 effects) and spin-lattice relaxation (T_1).

Spin-spin coupling

In liquids, the magnetic moments of the nuclei of a molecule can have interactions that are mediated by the electrons in the molecular bonds. These through-bond interactions are called spin-spin couplings. The spin-spin couplings give rise to a fine structure of the NMR resonances. Their magnitude is expressed in hertz (Hz). Modern NMR spectrometers are able to separate information about chemical shifts and spin-spin interactions in a two-dimensional NMR spectrum (Veeman 1997). In such a spectrum, the NMR frequencies are plotted along two axes. The spectrum is generally shown as the top view of the resulting "mountain landscape" of NMR lines. A two-dimensional approach strongly simplifies spectral interpretation and is applied to elucidate the structure of, e.g., complex proteins and DNA. By a careful analysis of the chemical shift information and spin-spin couplings, the NMR spectroscopist is able to determine the molecular structure of an unknown compound. This is the basis of the NMR finger print method that is commonly used in analytical applications.

An example of spin-spin coupling in a ^{13}C NMR spectrum is shown in Figure 2. For natural abundance ^{13}C NMR (1.11% of the total C pool), the probability that two ^{13}C nuclei interact is small. In this case, a ^{13}C NMR spectrum with only chemical shift information is obtained (e.g. A2 and B2 in Figure 2). However, ^{13}C-^{13}C spin-spin interactions can be observed in ^{13}C-enriched molecules (e.g., P2 and P3 in Figure 2).

NMR methods

The scope of NMR applications encompasses solutions, solids and intermediate physical states of pure materials or mixtures in which a wide range of magnetic nuclei of both inorganic and organic molecules can be probed.

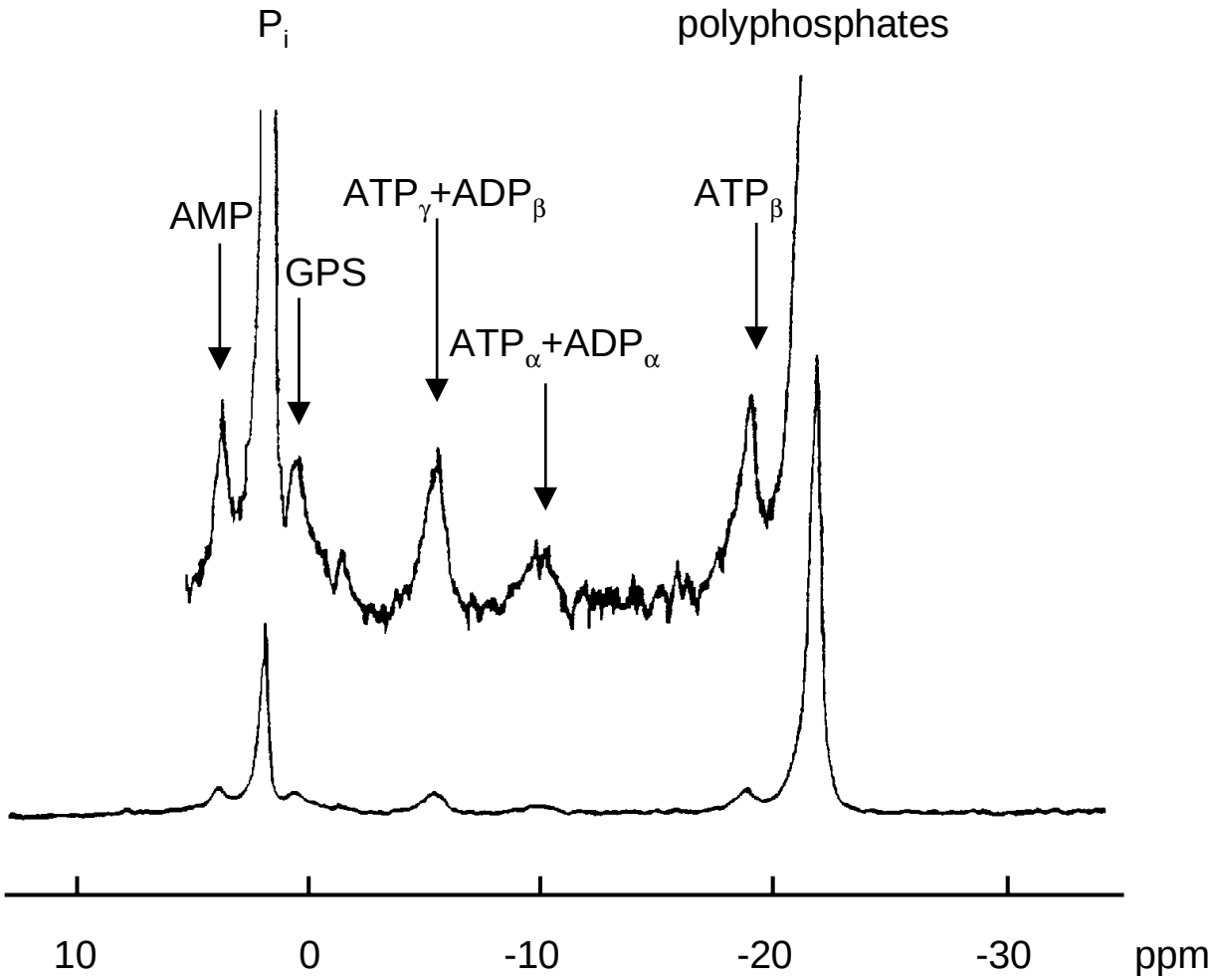

Figure 1. High resolution (162 MHz) ^{31}P NMR spectrum of activated sludge (after Florentz et al. 1984). Each peak in the spectrum shows a particular ^{31}P nucleus of the molecules present in the sample. The peaks are assigned in the insert (GPS: glycerol 3-phosphorylserine, P_i: inorganic phosphate). The location of the peaks on the ppm axis arises from the chemical shift differences, whereas their intensity is related to the relative concentration of the ^{31}P nuclei. This information can be used to unravel metabolic pathways of energy-rich compounds.

High-resolution solution NMR

In high-resolution NMR, a sample is ideally introduced into a NMR spectrometer with a high magnetic field to achieve the best resolution and signal-to-noise ratios. The magnetic field B_0 of superconducting electromagnets in modern NMR instruments ranges from 7.0–18.8 T. High-resolution NMR is particularly suited for liquid samples. In this way, isotopes with a low natural abundance or low sensitivity (Table 1) have become accessible to NMR study. Also samples containing microbial or plant cell cultures can be used, but the resolution of the spectra decreases due to line broadening effects of these heterogeneous samples (Fernandez & Clark 1987).

In environmental studies, high-resolution NMR is often used as a complementary technique to other chemical analytical methods, such as, e.g., mass spectroscopy, UV-visible, fluorescence, and infrared spectroscopy. In some cases, the information provided by NMR spectroscopy can aid in selecting appropriate methods for conventional chemical analysis of, for example, individual lipids, sugars or amino acids.

Solid state NMR

Solid samples which can be dissolved in solvents can be examined by high-resolution solution NMR. However, high-resolution solid state NMR allows to study directly the chemical structure of environmental

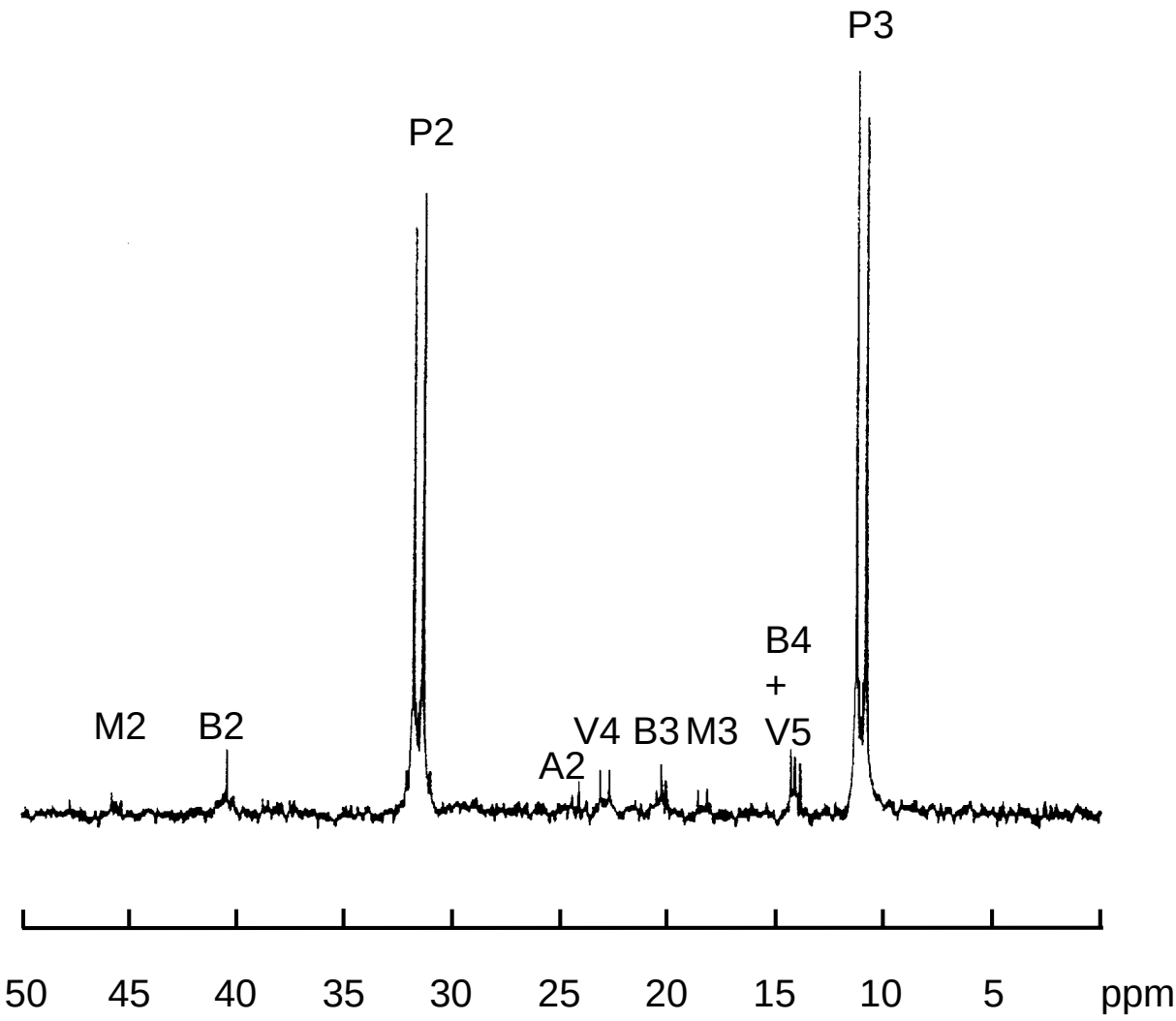

Figure 2. High resolution (300 MHz) ^{1}H-decoupled ^{13}C NMR spectrum showing the metabolisation of [2,3-^{13}C]propionate in the presence of butyrate by syntrophic anaerobic sludge (after Lens et al. 1996). A: Acetate; B: butyrate; M: Methyl-butyrate; P: Propionate and V: Valerate. The numbers following the one-letter abbreviations give the positions of the carbon in the molecule. This spectrum illustrates the spin-spin coupling between the C_2 and C_3 atom of propionate, which results in a characteristic splitting of 34 Hz. This typical distance allows to follow the fate of this molecular skeleton during bioconversion: [2,3-^{13}C]propionate is converted into [4,5-^{13}C]valerate and 2 methyl-[2,3-^{13}C]-butyrate. The characteristic splitting is useful to identify a resonance peak of a compound in case of low signal to noise ratio (e.g., M2) or overlap with the resonance peak of another atom (B4 and V5). Note that, under the experimental conditions applied, monolabeled compounds give almost overlapping resonance positions for the peaks of B4 (14.2 ppm) and V5 (14.1 ppm). The spin-spin coupling splits the V5 peak over 34 Hz around its resonance position, thus clearly discriminating the B4 and V5 peaks.

important solids. This type of NMR dates from the mid-1970s, when the first magic-angle sample spinning devices were built. Magic-angle-spinning (MAS) NMR is most useful for observing ^{13}C and ^{31}P nuclei in solids. The solid sample is rapidly rotated (in the order of kHz) in a spinner about an axis that makes an angle of 54°44′ (the so-called magic angle) with the static magnetic field B_0. This mechanical rotation dramatically reduces the effect of several line broadening interactions. Among these are the chemical shift anisotropy, which becomes important for materials that are static or in a very slow motion on the time scale of the NMR experiment (10^{-3} to 10^{-4} s), and nuclear dipole-dipole interactions. The problem of low sensitivity is tackled by a pulse sequence in MAS NMR that transfers the magnetization of the abundant protons to the ^{13}C, or ^{31}P nuclei, that generally have a much lower concentration in the

molecules. This method is called cross-polarization (CP) NMR. More details of applications of this type of NMR can be found in the reviews by Preston (1996), Kögel-Knabner (1997) and Kentgens (1997).

X-ray diffraction analysis is one of the traditional methods used to determine the crystalline nature of solid materials. However, when dealing with solids that are not highly ordered on a molecular level, X-ray diffraction analysis is not useful. For these amorphous solids, MAS NMR is particularly interesting for interpreting molecular structures.

Relaxation time measurements

Although the relaxation times T_1 and T_2 can be determined of separate nuclei in high-resolution NMR spectroscopy, relaxation studies are most frequently carried out on solvents, i.e., water.

Both relaxation times of water depend on the physical environment (adsorbing walls, packing density), and thus are utilised in geochemistry to characterise soils and sediments (Kenyon 1992). Also the chemical composition of the liquid determines both relaxation times. Especially the presence of paramagnetic ions (e.g., Fe, Mn and Gd) drastically changes T_1 and T_2, and thus are applied as contrast or masking agents (Nestle & Kimmich 1996a, 1996b). This has become important in NMR imaging studies (see below).

Magnetic resonance imaging (MRI)

In high-resolution NMR spectroscopy, a very homogeneous magnet is used to reduce line broadening effects, and the resulting NMR signals are monitored for the whole sample. In contrast in magnetic resonance imaging (MRI), the resulting signal is recorded for a sample subdivided in a number of picture (pixels) or volume (voxels) elements by the use of strong magnetic field gradients applied to the sample. The result is that MRI gives spatially resolved information about the sample, which can be obtained *in vivo* and non-invasively. For sensitivity reasons, MRI is only practically feasible for studying highly abundant nuclei, such as the water protons in a sample. Apart from MRI, various other names have been given to the imaging technique, including NMR imaging, spin imaging, spin mapping, NMR tomography and zeugmatography.

In general, the spatial resolution is at the expense of the signal-to-noise ratio per pixel. To date, a spatial resolution of up to 10 μm can be obtained for ^{1}H of water, thus enabling NMR microscopy of individual plant and mammalian cells (Aiken et al. 1995). However, for most environmental applications, e.g., sludges and soils, a spatial resolution of about 50–100 μm is more realistic.

Other NMR techniques

Although effects of flow and motion on the NMR signal are known for a long time, NMR has not been used very much for flow and diffusion measurements until recently. NMR is well capable of discriminating proton spins of flowing and stationary water on the basis of the physical properties of flow (Callaghan 1991). Flow results in a net displacement of the water protons, whereas the NMR signal of stationary water is still subjected to a random walk motion due to self diffusion. All NMR techniques that are used to measure transport properties employ magnetic field gradients on top of the externally applied steady magnetic field of the NMR magnet. Pulsed field gradient (PFG) NMR flow methods have been described in detail by Caprihan & Fukushima (1990), whereas PFG NMR techniques to determine self-diffusion coefficients are presented by Stilbs (1987). It is also possible to combine NMR flow measurements and imaging. An example of this approach in a bioreactor is shown in Figure 3.

Over the years, advances in understanding the intracellular environment of cells have been accompanied by increasing interest in the behaviour of enzymes *in situ.* Magnetisation transfer and isotope exchange can be applied to analyse, both *in vitro* and *in vivo*, enzyme catalysed reactions (Fernandez & Clark 1987). Both techniques involve targeting the chemical species of interest, e.g. the substrate of an enzymic reaction, and subsequent observation of these nuclei during the reaction sequence under consideration.

Applications of NMR in environmental engineering

NMR as a detection method

NMR was introduced as an advanced analytical tool for the determination of molecular structures in the 1960–1970s. One of the first reports of NMR in environmental engineering, actually a patent, indeed proposes to connect an NMR device at the outlet of an activated sludge unit to monitor its effluent quality (Petroff 1975).

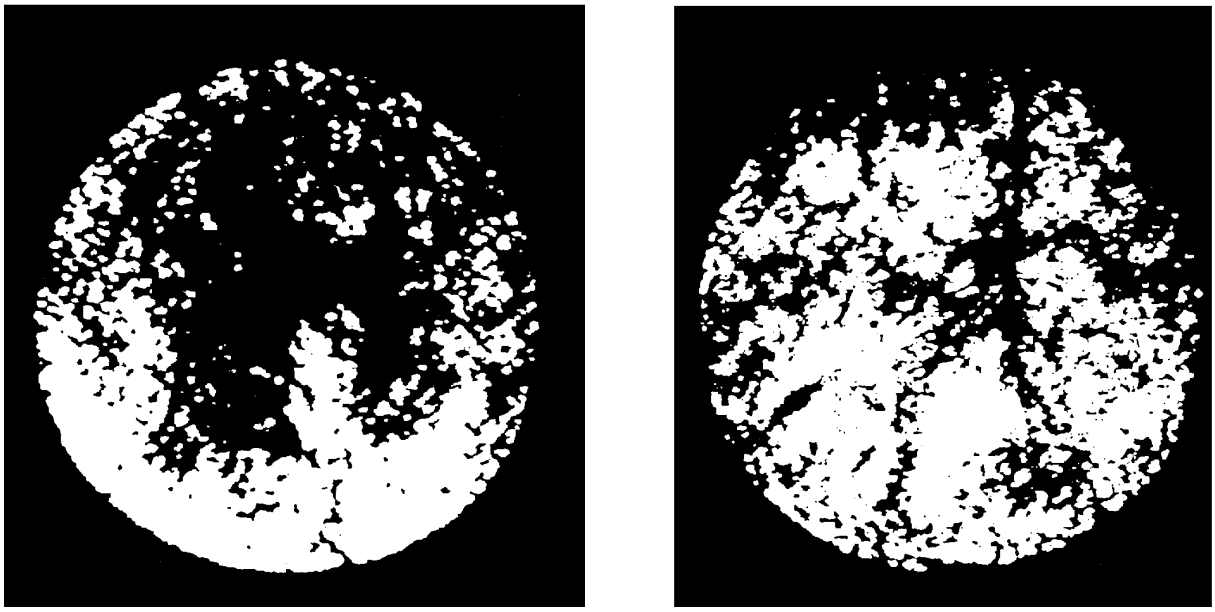

Figure 3. ^{1}H NMR images of two cross sections of flowing water in a hollow fibre bioreactor containing mammalian cells (after Donoghue 1992). The outer diameter of the reactor is 5 cm. In the images, the dark regions indicate flowing water, whereas the white regions represent domains of cell growth (no flow). In the image taken near the front of the reactor (left), a region of little or no growth is present in the centre of the reactor. Near the outlet (right), the region of cell growth extends further into the inner regions.

To date, NMR is still frequently used as a method to confirm the chemical structure of newly synthesised compounds or degradation products (Table 2). In this approach, NMR is often used in combination with other chemical analytical methods. A comparative study of NMR with various other techniques has been performed for paper mill wastewaters, landfill leachates, oil spills, pesticides, polychlorinated biphenyls and taste and odour causing substances (Keith & Hercules 1973).

Recently, NMR has been used to assess the capacity of different environmental samples to degrade xenobiotics (Castro et al. 1996). In this approach, addition of ^{13}C-labeled target compounds facilitated the screening of soil environments able to degrade those selected xenobiotic compounds.

NMR to study metabolic processes

NMR has been widely used to study the conversion and fate of pollutants during their abiotic (Table 3) or biotic (Tables 4–5) conversion. The advantage of NMR techniques is that they allow the continuous and non invasive observation of microbial (Chen & Bailey 1993), plant (Fernandez & Clark 1987), or mammalian (Gillies et al. 1989) cell cultures. Consequently, different types of NMR have been widely used to study the biotransformation and biodegradation pathways of organic compounds, including volatile fatty acids (Tholozan et al. 1988), cholesterol (Jadoun & Bar 1993), quaternary ammonium (Janosz-Rajczyk 1991) and linear alkyl sulphonate (Cavalli et al. 1996) detergents, di-isopropylnaphthalene (Yoshida & Kojima 1978) and chlorinated acetate (Egli et al. 1989). Moreover, also toxicity mechanisms of many polluting chemicals have been unravelled using NMR. For this aspect of cellular metabolism, ^{19}F-labeled compounds have been used as well.

NMR is a unique tool in metabolic studies, as it allows to follow the fate of each atom of a molecule during the bioconversion. Typical examples of NMR applications are propionate scrambling in the succinate pathway (Tholozan et al. 1988) or isomerisation of butyrate into isobutyrate (Oude Elferink et al. 1996). In both cases, enzymatic reactions of anaerobic bacteria rearrange the substrate molecule, i.e., [3-^{13}C]-propionate is converted into [2-^{13}C]-propionate during scrambling and [2-^{13}C]-butyrate is isomerised into [3,4-^{13}C]-isobutyrate. Study of these molecular interconversions are only accessible by use of labeled substrates. They are elegantly accessible by NMR using magnetic nuclei, which avoid the safety regulations

Table 2. Use of NMR as an analytical tool in environmental engineering

Application	Technique	Reference
Wastewater characterisation		
Unbleached kraft pulp	^{13}C NMR	Mikama et al. 1995
Fulvic acids in drinking water treatment	^{13}C NMR	Shin & Lim 1996
Off gas scrubbing wastewater	^{1}H NMR	Deutsch et al. 1979
Determination of inorganic phosphates	^{31}P NMR	Gurley & Ritchey 1975
Speciation of phosphorous species	^{31}P NMR on extracts	Schoenborn 1995
Characterisation of functional compounds		
Biofilm forming polysaccharides of *Staphylococcus epidermis*	^{1}H NMR	Mack et al. 1996
Osmoregulating proteins in *Salmonella manhattan*	^{1}H NMR	Dupray et al. 1995
Characterisation of storage products/polymers		
Biosynthesis of polyhydroxybutyrate	^{13}C NMR	Doi et al. 1990
Microstructure of copoly(3-hydroxyalkanoates)	^{13}C NMR	Inoue et al. 1996
Characterisation of filtration media		
Zeolites	^{129}Xe NMR	Stöcker 1996
Peat	^{1}H NMR	Rasmussen et al. 1996
Porous rocks and soils	^{1}H relaxation times	Kenyon 1992; Kleinberg 1994

Table 3. Applications of NMR to study physico-chemical treatment methods

Physico-chemical treatment	Substrate	Technique	Reference
Ozonisation	Bromacil	^{1}H and ^{13}C NMR	Archer et al. 1994
Chlorination	House boat wastewater	^{13}C NMR	Adams et al. 1975
Pyrolysis	Car shredder residue	^{1}H NMR	Lin et al. 1996
Photooxidation	Daminozide	^{1}H and ^{13}C NMR	Brown & Casida 1988
Photooxidation and H_2O_2 (Fenton pretreatment)	Anthraquinone sulfonate	^{1}H NMR	Kiwi et al. 1993

and disposal problems associated with radioactively labeled compounds.

The most commonly used techniques to study bioconversion and biodegradation are ^{31}P, ^{13}C and ^{1}H NMR spectroscopy. The ^{31}P nucleus has a 100% natural abundance (Table 1) and is thus excellently suited for NMR analysis. ^{31}P NMR can be used to determine the intra- and extracellular concentrations of phosphorylated metabolites, e.g., polyphosphates, phospho monoesters, sugar phosphates, inorganic phosphate, NAD and ATP (Fernandez & Clark 1987). Moreover, ^{31}P NMR spectroscopy enables the *in vivo* measurement of the intracellular pH and is thus an excellent tool for the *in vivo* analysis of metabolic pathways and cellular regulation mechanisms. Consequently, ^{31}P NMR has been extensively applied in a wide range of environmental engineering issues (Tables 4–5).

The use of ^{13}C-labeled substrates permits the quantification of mass flows over different metabolic pathways (Zupke & Foy 1995). As only about 1% of the carbon atoms have magnetic properties (Table 1), and are accessible to NMR measurements, a disadvantage of ^{13}C NMR spectroscopy is its relatively low sensitivity. Detection of intermediates can be facilitated by prolonging the measuring time, using concentrated cell cultures, and using ^{13}C-labeled substrates. If ^{13}C-labeled compounds are used, one can also consider the use of double labeled compounds (see Figure 2 for an example). In these compounds, spin-spin interactions split the resonance peaks in two with a characteristic splitting of 34 and 52 Hz in case of a carboxyl and carboxylic acid neighbouring atom, respectively. These typical distances in a ^{13}C NMR spectrum allow to follow, for example, how a molecular skeleton is

Table 4. Environmental engineering applications of ^{31}P NMR spectroscopy

Application	Microorganism/sludge type	Reference
Speciation of P compounds in sludges		
P-fractionation	Waste activated sludge, after aerobic and anaerobic digestion	Hinedi et al. 1989
P-fractionation	Biological P removing activated sludge	Uhlmann et al. 1990
Mineral forms of P	Activated sludge	Frossard et al. 1994a
P removal		
Chemical P removal	Amorphous P-Al crystals	Duffy & vanLoon 1995
NMR of poly-P polymers	Activated sludge	Roeske & Schoenborn 1994
Biological P removal	Meat extract treating activated sludge	Florentz & Granger 1983; Florentz et al. 1984
Biological P removal	Glucose and starch treating activated sludge	Hill et al. 1989; Jing et al. 1992
pH determination		
Intracellular pH	Yeast	Melvin & Shanks 1996
Toxicity studies		
Inhibitory effect of N-serve on nitrification	Nitrifying activated sludge	Benmoussa et al. 1984
Inhibitory effect of NO_2^-	*Pseudomonas*	Sijbesma et al. 1996

Table 5. Bioreactor designs used for the *in vivo* NMR of high cell densities

Bioreactor type	Organism cultivated	Technique	Reference
Stirred tank reactor	Potato spindle tuber viroid	^{31}P NMR	Berlin et al. 1985
Continuous flow	*Zymomonas mobilis*	^{31}P NMR	De Graaf et al. 1992
Membrane cyclone	*Zymomonas mobilis*; *Corynebacterium glutamicum*	^{31}P and ^{13}C NMR	Hartbrich et al. 1996
Airlift bioreactor	*Aspergillus terreus*	^{31}P NMR	Lyngstad & Grasdalen 1993; Melvin & Shanks 1996
Fluidized bed	Mammalian cells	Localised ^{31}PNMR	Schuppenhauer et al. 1995
Hollow fiber	*E. coli*, *Sacharomyces*	^{23}Na MRI	DiBiasio et al. 1993
Multi tube hollow fibre	Mammalian cells	^{1}H MRI	Donoghue et al. 1992

incorporated into newly synthesised products (Lens et al. 1996).

For certain applications, additional ^{1}H NMR measurements are performed to confirm the results of the ^{13}C NMR spectra. Although the ^{1}H nucleus has a 100% abundance, ^{1}H NMR spectroscopy can be limited by the concentration of the compound under investigation. If the concentration of the compound is too low, its ^{1}H NMR signal can be masked by the ^{1}H NMR signal of the extremely abundant water (or solvent) phase.

The use of concentrated cell suspensions or cell extracts is an elegant way to obtain intermediates in higher concentrations, especially for the study of anaerobic conversion processes. However, it permits only limited conclusions to be drawn concerning metabolic processes under *in vivo* conditions, because the physiology of microorganisms can change during the cell harvest and sample preparation (Preston 1996) or during the relatively long measuring time of NMR spectroscopy. For this reason, a variety of small reactor systems has been recently developed that permit *in situ* cultivation of microorganisms and continuous fermentation within the NMR magnet (Table 5). Thus, the intensity of the NMR signals of intracellular metabolites can be increased by concentrating the cell mass in a reactor system. Moreover, the microorgan-

isms can be kept in a well-defined, stable metabolic state over a prolonged period of time.

An important limiting factor in high cell density fermentations is the supply of gaseous substrates, i.e., oxygen and methane for aerobic and methanotrophic microorganisms, respectively. Conventional reactor systems might have an insufficient gas/liquid transfer for these low soluble gases. For example, up to 7 g O_2/l.h can be fed into conventional stirred tank reactors, whereas a cell mass of 50 g/l dry weight of the aerobic microorganism *Corynebacterium glutamicum* has an oxygen demand of up to 15 g O_2/l.h (Büchs 1988). The application of advanced bioreactor aeration principles in the NMR probe, e.g., cyclone aeration, successfully have overcome the mass transfer limitation of the gaseous substrates (Hartbrich et al. 1996).

Biological nutrient removal

Different types of NMR have been applied to study both chemical and biological phosphorous removal (Table 4). ^{31}P NMR in combination with ^{27}Al NMR was used to study amorphous precipitates formed during alum and aluminum derivate addition for physicochemical phosphorous removal. Because of the importance of the information on bioenergetics provided by ^{31}P NMR, considerable effort has been given to elucidate the biochemistry of biological phosphorous removal using NMR. ^{31}P NMR has been applied to localise the polyphosphates within bacterial cells (Hill et al. 1989) and to study the effect of the carbon source (Jing et al. 1992), or the length of the aerobic and anaerobic phase (Florentz et al. 1984) on the efficiency of phosphorous removal. By using *in vivo* ^{31}P NMR in combination with ^{13}C NMR, a new metabolic model for the biological phosphorous removal has been developed (Pereira et al. 1996). The NMR data unequivocally showed that ^{13}C-labeled acetate is incorporated in polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) during the anaerobic stage. Both PHB and PHV are subsequently converted to glycogen in the aerobic stage. Pereira et al. (1996) further used the NMR data to quantify the reaction stoichiometry of these metabolic routes.

Due to the low natural abundance of the ^{15}N isotope and its poor magnetic properties (low and negative gyromagnetic ratio), the sensitivity of a ^{15}N NMR experiment is approximately 50 times lower than for a ^{13}C NMR experiment (Table 1). This may be the reason why ^{15}N NMR on environmental samples with natural ^{15}N levels was previously thought impossible. Recent studies, applying optimised ^{15}N NMR spectroscopic parameters, showed that solid state ^{15}N NMR spectra of soils can be obtained with an acceptable signal-to-noise ratio, if their organic nitrogen content exceeds 1% (Knicker & Lüdemann 1995). Such concentrations are not commonly found in solid wastes or polluted soils, excluding such samples from ^{15}N NMR spectroscopic investigations. However, nitrogen conversions and their dynamics can be studied by NMR in extremely polluted environments, or if ^{15}N-enriched materials are used (Knicker et al. 1996).

Similar as the naturally abundant oxygen isotope (^{16}O), also the naturally abundant sulfur isotope (^{32}S) has no spin and related magnetic properties, and thus direct NMR spectroscopic techniques can not be applied. However, the metabolism of bacteria involved in biological sulfur removal has been studied indirectly by monitoring the fate of ^{13}C-labeled compounds in the presence of sulfur compounds (Omil et al. 1997; Lens et al. 1998). Using ^{13}C-enriched propionate, sulfate reducing bacteria present in a biological sulfate reducing reactor were shown to follow the succinate pathway during propionate conversion (Omil et al. 1997). Similarly, the metabolic properties of sulfide oxidising sludge were investigated and the presence of strictly anaerobic sulfate or sulfur reducing bacteria in these microaerobic reactors was demonstrated by NMR. Also in the sulfide oxidising sludge, the succinate pathway was the major ^{13}C-propionate degradation pathway (Lens et al. 1998).

Heavy metal removal

Some metals often associated with pollution, i.e., ^{27}Al, ^{65}Zn and ^{113}Cd, possess magnetic properties and thus can be probed directly by NMR. Thus far, however, relatively little NMR investigations are reported on the removal or processing of heavy metals. ^{113}Cd NMR spectroscopy has been used to probe metal binding sites in algae (Ke & Rayson 1990), humic acids (Chung & Moon 1994) and montmorillonite clay (Jun et al. 1996). Moreover, ^{113}Cd NMR has been applied to investigate the effect of Cd pollution on enzyme systems, such as, e.g., metallothioneins (Vašák 1998). Ogoma et al. (1992) compared the binding capacity of proteins for the metals ^{65}Zn and ^{25}Mg to that for ^{43}Ca and ^{29}K by direct NMR spectroscopy. ^{27}Al NMR has been used to investigate phosphorous precipitates (Duffy & vanLoon 1995) and zeolite architecture (Stöcker 1996).

Apart from direct NMR of these metal nuclei, also their paramagnetic effects can be probed indirectly by NMR via relaxation time measurements of the aqueous solvent. Many heavy metals of environmental relevance, i.e., Fe^{2+}, Mn^{2+}, Cu^{2+}, Cr^{3+}, Gd^{3+}, VO^{2+} have paramagnetic properties. These paramagnetic ions reduce the T_1 or T_2 relaxation rates of their solvent. Thus, relaxation time measurements allow to monitor the transport of paramagnetic heavy metals within a biofilm, sludge, solid waste, or soil. A detailed study of the mechanisms of heavy metal penetration using these NMR procedures in alginate, immobilized cells and Kombu algal biosorbents was reported by Nestle and Kimmich (1996a, 1996b). These authors also imaged the penetration of heavy metals in these organic matrixes, which opens perspectives for dynamic studies of heavy metal transport in biofilms, or the study of the mechanisms of microbial-induced corrosion.

Solid waste and sludge treatment

NMR has been used to assist the environmental engineer in the study and optimisation of solid waste and sludge treatment (Table 6). The organic fraction present in solid waste is very diverse and difficult to define. NMR analysis of extracts can be used to monitor changes in organic matter content during the treatment process. In general, high concentrations of organic matter are present in the extracts, making the use of ^{13}C-labeled substrates redundant.

NMR can also be performed directly on the solid waste or sludge using solid state NMR techniques, i.e., CP MAS ^{13}C NMR (Inbar et al. 1991; 1992). This technique is not restricted to ^{13}C compounds, and also CP MAS ^{31}P and ^{27}Al NMR has been applied in the study of chemical waste sludges (Duffy and vanLoon 1995).

In aqueous solutions, the amplitude of the ^{1}H NMR signal of water protons can be considered as a direct measure of the amount of water present in a sludge sample. Thus, ^{1}H NMR can be used to determine the hydration state of solid wastes or sludges as a function of the drying process (Sato et al. 1980; La Heij et al. 1996). Additional information can be obtained about the water status of solid wastes when ^{1}H NMR amplitude measurements are performed spatially resolved (imaging) or combined with relaxation time or diffusion measurements (see below). The same imaging procedures can be applied to study the fate and transport of other polluting ^{1}H containing mate-

Table 6. Applications of NMR used in the treatment and utilisation of solid waste and sludges

Application	Solid waste/sludge type	Technique	Reference
Solid waste			
Effect of humic substances on composting	Wine solid	^{13}C CP MAS NMR	Inbar et al. 1991; 1992
Evaluation of inhibitory compounds	Coffee residues	^{13}C and ^{1}H NMR	Nagaoka et al. 1996
Characterisation liquefaction products	Municipal solid waste	^{13}C NMR	Gharieb et al. 1995
Characterisation aromatic fraction	Tar, coal	^{13}C NMR and CP MAS NMR	Maroto-Valer et al. 1996
Wastewater treatment sludges			
Evaluation of inhibitory compounds	Activated sludge	^{13}C and ^{1}H NMR	Nagaoka et al. 1996
Fate of P during fertilisation	Activated sludge	^{31}P and ^{13}C NMR	Frossard et al. 1994b
Characterisation of Al precipitates during P removal	Al dosed activated sludge	^{27}Al and ^{31}P CP MAS	Duffy & vanLoon 1995
Hydration state	Activated sludge; Aluminium hydroxide	^{1}H NMR	Sato et al. 1980
Sludge dewatering	Activated sludge	T_1 and T_2 relaxation of ^{1}H, ^{1}H MRI	La Heij et al. 1996
Water fractions	Anaerobic granular sludge	T_2 relaxation of ^{1}H	Lens et al. 1997

rials, such as solvents, oil and hydrocarbons in solid wastes and sludges.

Soil pollution

Applications of NMR for the study for various processes in natural soils have been reviewed recently (Kögel-Knabner 1997; Preston 1996). For more general reading, the reader is referred to a recent special issue about NMR in soil science (Hemminga & Buurman 1997). The great strength of NMR to study transformations of soil organic matter (SOM) is its unique ability to provide information on more complex materials, i.e. those characterised by lower solubility, irregular structures and strong physical or chemical links to each other or to mineral matter (Wais et al. 1995). One question associated inevitably with SOM studies is whether to work with intact soil or to fractionate, and if so, how. The early emphasis on humic and fulvic acids was in part driven by the need to fractionate SOM in forms that were amenable to the chemical and spectroscopic techniques available. Nowadays, humic and fulvic acids can be measured by NMR, not as the prime representatives of SOM, but as part of a spectrum of physical and chemical fractions present in the NMR spectrum. Consequently, ^{13}C NMR has been successfully applied to understand decomposition processes and characterise plant biopolymers in soils (Preston 1996).

Naturally occurring soil materials, both organic and inorganic, are generally poor specimens for direct NMR study for two reasons. First, transition metal ions (mostly Fe and Mn) are an ubiquitous interference. Even though one may see a signal in the NMR spectrum of a natural sample, many of the chemical environments will not appear because of severe paramagnetic line broadening. Second, natural materials are a mixture of many types of chemical environments. Line broadening due to chemical heterogeneity severely reduces resolution and cannot be removed by MAS or technologically accessible field strengths. CP MAS ^{13}C NMR can, nevertheless, detect non-natural, polluting organic additives to soil, as, e.g., brown coal emissions from a briquette factory (Schmidt et al. 1997) or airborne lignite particles from open cast mining (Rumpel et al. 1996). Also the interactions between oil residues and sediments can be characterised by solid state ^{13}C NMR spectroscopy and ^{1}H imaging NMR (Chudek & Reeves 1998).

NMR can also be used to characterise the structure of small defined molecules, e.g. pesticides or their metabolites, that closely associate with SOM or the complex soil matrix upon their release in the soil (Kögel-Knabner 1997). Because of its relatively low sensitivity, NMR is unlikely to displace well-established analytical techniques such as high pressure liquid chromatography (HPLC) or gas chromatography (GC) for routine applications. However, NMR can be of great advantage in understanding the behaviour of xenobiotics in soil, in the development of novel analytical techniques and in special circumstances where conventional separation or detection techniques do not work well. To date, using ^{13}C-labeled pollutants, NMR clearly resolved the fate of the herbicide 2,4-dichlorophenol (Hatcher et al. 1993; Nanny et al. 1996), the fungicide anilazine (Haider et al. 1993) and the organic pollutants acetone, trichloroethylene and carbon tetrachloride (Jurkiewicz & Maciel 1995) in soils.

Bioreactor hydrodynamics

In the last ten years, various microscale (8–84 ml) bioreactor designs have been used in ^{13}C and ^{31}P NMR studies of dense cell cultures (Table 5). The NMR tools available can also be used to study much larger (up to 20 l) bioreactors, as reviewed by Gillies et al. (1989) and Clark & Fernandez (1991).

Interestingly, apart from the characterisation of metabolic properties, NMR also offers the possibility to study transport processes, i.e., diffusion and flow, in bioreactors. A major attraction of NMR is that it is non-invasive, so that no direct contact with the fluid is necessary. Hence, it is well suited for studies of liquids that need to be isolated, such as those having extreme temperature, chemical reactivity or are abrasive. NMR does not use ionizing radiation, in contrast to X-ray scattering flow methods. Another special property of NMR is that flow can be detected in any direction within the sample, in contrast to X-ray, optical and ultra-sound scattering flow methods which only measure a net flow between the emitter and the detector.

At this moment, measurements of transport phenomena by PFG NMR are restricted to protons, thus enabling detailed studies of water transport processes in pipes and model systems (Caprihan & Fukushima 1990), polystyrene beads (Tallarek et al. 1996) and soils (Van As & Van Dusschoten 1997). Also water flow in biofilm reactors has been studied using NMR, i.e. in aerobic *Pseudomonas* biofilm systems (Lewandowski et al. 1994) and sulfidogenic granular

sludge reactors (Lens et al. 1997). PFG NMR is not restricted to water transport, and has also been applied to study transport of oily emulsions and light oil in porous rocks (Mardon et al. 1996) and nonaqueous phase liquid (NAPL) in a simulated pump and treat remediation process (Gladden 1996).

Further developments and perspectives

Use of LC-NMR to increase the analytical resolution of NMR

One of the problems with the application of analytical techniques in the field of environmental science is the complex nature of the samples. For example, in biodegradation processes several compounds are present that need to be identified. As described above, this problem is often overcome by the utilisation of specific isotopes, such as ^{13}C and ^{19}F. Alternatively, the abundant isotope ^{31}P is used to monitor phosphorous-containing compounds. Also two-dimensional NMR techniques have contributed to an improved assignment of resonances of complex samples. However, there is still an increasing need to facilitate the analysis of the composition of complex mixtures, and the methods mentioned above can not always be used. For this reason, there has been a rapid progress in the coupling of a chromatographic system to the NMR spectrometer. This is called liquid chromatography (LC) NMR. Individual components of the mixture can be analysed by NMR in a stopped-flow or on-flow mode of the LC column. As compared to off-line measurements, drastic time savings and new information about the sample can be obtained. Because the NMR technique is non-destructive, the sample can be saved in a fraction collector for other analytical purposes. For example, it may be expected that an additional coupling of an ion-trap mass spectrometer to an LC-NMR system will provide possibilities for a fully automated analysis of complex mixtures.

Several recent technical improvements have considerably increased the detection limit of LC-NMR systems. With the introduction of high-field spectrometers (800 MHz for ^{1}H NMR), an increased level of spectral quality is obtained in terms of sensitivity and spectral resolution. The analytical power of LC-NMR systems will further grow by improvements of probe head designs with “capillary” coils and the development of cryo-probes, operating at 77 K. These system modifications can substantially decrease the detection limit of LC NMR systems. In addition, improved NMR data processing by multicomponent analytical data analysis methods, pattern recognition procedures, and process automation will further increase the resolution of the obtained NMR information (Nicholson & Wilson 1989).

Spectroscopy of other nuclei and electrons

NMR applications in environmental science and engineering are not limited to the applications discussed in this review. Actually, any nucleus with magnetic properties can be probed directly by NMR. Some environmental relevant nuclei for which NMR spectroscopy has been applied are ^{7}Li (Abraha et al. 1991), ^{11}B (Singh et al. 1990), ^{23}Na (DiBiasio et al. 1993), ^{39}K (Ciulla et al. 1994) and ^{77}Se (Boles et al. 1992). NMR protocols are also available for the metal ^{195}Pt (Bancroft et al. 1990) and even the radioactive isotope ^{133}Cs (Li et al. 1995). NMR spectroscopy and imaging of these nuclei can assist in fundamental or applied studies of the fate, sorption and transformation of compounds that contain these nuclei.

In this paper, several applications of NMR have been presented. In some cases, however, electron spin resonance (ESR) can be used as well to study the reaction mechanism of interest (Kiwi et al. 1993). An intriguing application of ESR is the study of reaction mechanisms mediated by radicals. This opens perspectives to study radical utilising physico-chemical (ozonisation, Fenton reactions, photochemical oxidation) and biological (fungal degradation) waste treatment methods.

Bioreactor analysis

Coupling flow and/or self-diffusion measurements to the determination of the relaxation time T_2 (Van Dusschoten et al. 1996), enables to discriminate the motional behaviour of the different ^{1}H ensembles present in a bioreactor system. Thus, flow and dispersion of different ^{1}H fractions can be determined in various bioreactor and soil types (Van As & van Dusschoten 1997). These NMR procedures can also be used in combination with imaging and localized spectroscopy techniques to monitor the growth and distribution of cells in a reactor system. When coupling these non-invasive ^{1}H NMR measurements to ^{31}P NMR, this type of NMR also allows to investigate metabolic heterogeneity within a reactor and can assist in future design of bioreactor systems.

Relaxation time measurements have also been applied to make temperature images of biological tissues (Young et al. 1994). This NMR application is used in medicine to monitor temperature changes in real time during tissue heating (hyperthermia, laser surgery, focused ultrasounds) or cooling (cryotherapy). The method relies on the effect of temperature on the relaxation rates, which can be quantified using a calibration curve. Thus, NMR can assists in the design of heat exchangers or temperature phased bioreactors, e.g., membrane reactors (Ogawa 1998, personal communication).

Solid state NMR spectroscopy is nowadays a well-established technique used for structure elucidation and to describe the pore architecture, catalytic behaviour and mobility properties (like diffusion) of zeolites (Stöcker 1996). Important framework elements such as ^{27}Al, ^{29}Si and ^{31}P can be studied directly by NMR. Also, ^{129}Xe is a very suitable and sensitive isotope for probing the pore architecture of zeolitic materials. Much of the work performed with zeolites can be applied to other porous filtration or absorption/adsorption materials, e.g., ion exchange resins, peat and activated carbon, irrespective of their engineering application. It may be expected that NMR research will contribute to a better design of filtration reactor systems.

Acknowledgement

This research was supported in part by the European Community activity Large-Scale Facility Wageningen NMR Centre (ERBCHGECT940061) and a European Community Marie Curie fellowship (ERBFMBICT950250).

References

Abraha A, Mota de Freitas DE, Margarida M, Castro CA & Geraldes CFGC (1991) Competition between lithium and magnesium for ATP and ADP in aqueous solution: a multinuclear NMR study. J. Inorg. Biochem. 42: 191–198

Archer AJ, Hapeman CJ, Shelton DR, Muldoon MT, Lusby WR, Avni A & Waters R (1994) Comparison of formation and biodegradation of bromacil oxidation products in aqueous solutions. J. Agric. Food Chem. 42: 2040–2047

Adams VD, Middlebrooks EJ & Nance PD (1975) Organic residue in a recycled effluent. II. Water Sewage Works 122: 98–99

Aiken NR, Hsu EW & Blackband SJ (1995) A review of NMR microimaging studies of single cells. J. Magn. Reson. Anal. 1: 41–48

Bancroft DP, Lepre CA & Lippard SJ (1990) Platinum-195 NMR kinetic and mechanistic studies of cis- and trans-diamminedichloroplatinum(II) binding to DNA. J. Am. Chem. Soc. 112: 6860–6871

Benmoussa H, Fortin MN & Martin G (1984) Effect of a specific inhibitor of the nitrification: 2-chloro 6-trichloromethyl pyridin (N-serve). Rev. Fr. Sci. Eau 3: 137–146

Berlin J, Wray V, Forche E, Reng HG, Schueler H, Luckinger R & Muehlbach HP (1985) Production of potato spindle tuber viroid (PSTV) by large scale fermentation of PSTV-infected potato cell suspension cultures. J. Exp. Bot. 36: 1985–1995

Boles JO, Tolleson WH, Schmidt JC, Dunlap RB & Odom JD (1992) Selenomethionyl dihydrofolate reductase from *Escherichia coli*. Comparative biochemistry and ^{77}Se nuclear magnetic resonance spectroscopy. J. Biol. Chem. 267: 22217–22223

Brown MA & Casida JE (1988) Daminozide: oxidation by photochemically generated singlet oxygen to dimethylnitrosamine and succinic anhydride. J. Agric. Food Chem. 36: 1064–1066

Büchs J (1988) Immobilisierung von aeroben Mikroorganismen an Glassintermaterial am beispiel L-Leucin-Produktion mit *Corynebacterium glutamicum.* Ph.D. thesis, T.U. Hamburg-Harburg, Hamburg, Germany

Callaghan PT (1991) Principles of Nuclear Magnetic Resonance Microscopy. Clarendon Press, Oxford

Caprihan A & Fukushima E (1990) Flow measurements by NMR. Physics reports (review section on physics letters) 198: 195–235

Castro CE, O'Shea SK, Wang W & Bartnicki EW (1996) ^{13}C-NMR reactivity probes for the environment. Environ. Sci. Technol. 30: 1185–1191

Cavalli L, Cassani G, Lazzarin M, Maraschin C, Nucci G, Berna JL, Bravo J, Ferrer J & Moreno A (1996) Iso-branching of linear alkylbenzene sulfonate (LAS). Biodegradation study of two model compounds. Toxicol. Environ. Chem. 54: 167–186

Chudek JA & Reeves AD (1998) An application of nuclear magnetic resonance imaging to study migration rates of oil-related residues in estuarine sediments. Biodegradation 9: 443–449

Chen R & Bailey JE (1993) Observations of aerobic, growing *Escherichia coli* metabolism using an *on-line* nuclear magnetic resonance spectroscopy system. Biotechnol. Bioeng. 42: 215–221

Chung KH & Moon CH (1994) Selective binding of Cd^{2+} by natural and synthetic organic macromolecules investigated by ^{113}Cd NMR spectroscopy. Environ. Technol. 15: 795–800

Ciulla R, Clougherty C, Belay N, Krishnan S, Zhou C, Byrd D & Roberts MF (1994) Halotolerance of *Methanobacterium thermoautotrophicum* DELTA H and Marburg. J. Bacteriol. 176: 3177–3187

Clark DS & Fernandez EJ (1991) Noninvasive studies of bioreactors. Chemtech 21: 99–105

De Graaf AA, Wittig RM, Probst U, Strohhaecker J, Schoberth SM & Sahm H (1992) Continuous-flow NMR bioreactor for *in vivo* studies of microbial cell suspensions with low biomass concentrations. J. Magn. Reson. 98: 654–659

Deutsch K, Schimke D, Neugebauer B, Bielicki KH & Stoecker J (1979) Identification and determination of by-products of the acrylonitrile synthesis. J. Prakt. Chem. 321: 137–140

DiBiasio D, Scott J, Harris P & Moore S (1993) The relationship between fluid flow and cell growth in hollow-fiber bioreactors: applications of magnetic resonance imaging. BHR Group Conf. Ser. Publ. 5: 457–473

Doi Y, Segawa A & Kunioka M (1990) Biosynthesis and characterization of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) in *Alcaligenes eutrophus.* Int. J. Biol. Macromol. 12: 106–111

Donoghue C, Briedeau M, Newcomer P, Pangrle B, DiBasio D, Walsh E & Moore S (1992) Use of magnetic resonance imaging to analyze the performance of hollow-fiber bioreactors. Ann. N. Y. Acad. Sci. 665: 285–300

Duffy SJ & vanLoon GW (1995) Investigations of aluminum hydroxyphosphates and activated sludge by ^{27}Al and ^{31}P MAS NMR. Can. J. Chem. 73: 1645–1659

Dupray E, Derrien A & Pichon R (1995) Osmoregulation by trehalose synthesis in *Salmonella manhattan* after exposure to waste waters. Lett. Appl. Microbiol. 20: 148–151

Egli C, Thueer M, Suter D, Cook AM & Leisinger T (1989) Monochloro- and dichloroacetic acids as carbon and energy sources for a stable, methanogenic mixed culture. Arch. Microbiol. 152: 218–223

Fernandez EJ & Clark DS (1987) N.m.r. spectroscopy: a non-invasive tool for studying intracellular processes. Enzyme Microb. Technol. 9: 259–271

Florentz M & Granger P (1983) ^{31}P nuclear magnetic resonance of activated sludge: use for the study of the biological removal of phosphates from wastewater. Environ. Technol. Lett. 4: 9–14

Florentz M, Granger P & Hartemann P (1984) Use of ^{31}P nuclear magnetic resonance spectroscopy and electron microscopy to study phosphorus metabolism of microorganisms from wastewaters. Appl. Environ. Microbiol. 47: 519–525

Frossard E, Tekely P & Grimal JY (1994a) Characterization of phosphate species in urban sewage sludges by high-resolution solid-state ^{31}P NMR. Eur. J. Soil Sci. 45: 403–408

Frossard E, Tekely P & Morel JL (1994b) Chemical characterization and agronomic effectiveness of phosphorus applied as a polyphosphate-chitosan complex. Fert. Res. 37: 151–158

Gharieb HK, Faramawy S & El-Amrousi FA (1995) Liquefaction of cellulosic wastes. IV. Influence of inorganic catalysts and carrier oils. Fuel Sci. Technol. Int. 13: 393–411

Gillies RJ, MacKenzie NE & Dale BE (1989) Analyses of bioreactor performance by nuclear magnetic resonance spectroscopy. Bio/Technology 7: 50–54

Gladden LF (1996) Structure-transport relationships in porous media. Magnetic Resonance Imaging 14: 719–726

Gurley TW & Ritchey WM (1975) ^{31}P Fourier transform nuclear magnetic resonance spectrometry as a trace analysis tool for the determination of inorganic phosphates. Anal. Chem. 47: 1444–1446

Haider K, Spiteller M, Wais A & Fild M (1993) Evaluation of the binding mechanism of anilazine and its metabolites in soil metabolites in soil organic matter. Intern. J. Environ. Anal. Chem. 53: 125–137

Hartbrich A, Schmitz G, Weuster-Botz D, de Graaf AA & Wandrey C (1996) Development and application of a membrane cyclone reactor for *in vivo* NMR spectroscopy with high microbial cell densities. Biotechnol. Bioeng. 51: 624–635

Hatcher PG, Bortiatynski JM, Minard RD, Dec J & Bollag J-M (1993) Use of high-resolution ^{13}C NMR to examine the enzymatic covalent binding of ^{13}C-labeled 2,4-dichlorophenol to humic substances. Environ. Sci. Technol. 27: 2098–2103

Hemminga MA (1992) Introduction to NMR. Trends in foods Science & Technology 3: 179–186

Hemminga MA & Buurman P (1997) Special issue 'NMR in soil science'. Geoderma 80: 221–464

Hill WE, Benefield LD & Jing SR (1989) ^{31}P NMR spectroscopy characterization of polyphosphates in activated sludge exhibiting enhanced phosphorus removal. Water Res. 23: 1177–1181

Hinedi ZR, Chang AC & Lee RWK (1989) Characterization of phosphorus in sludge extracts using ^{31}P nuclear magnetic resonance spectroscopy. J. Environ. Qual. 18: 323–329

Inbar Y, Chen Y & Hadar Y (1991) ^{13}C CPMAS NMR and FTIR spectroscopic analysis of organic matter transformations during composting of solid wastes from wineries. Soil Sci. 152: 272–282

Inbar Y, Hadar Y & Chen Y (1992) Characterization of humic substances formed during the composting of solid wastes from wineries. Sci. Total Environ. 113: 35–48

Inoue Y, Sano F, Nakamura K, Yoshie N, Saito Y, Satoh H, Mino T & Matsuo T & Doi Y (1996) Microstructure of copoly(3-hydroxyalkanoates) produced in the anaerobic-aerobic activated sludge process. Polym. Int. 39: 183–189

Jadoun J & Bar R (1993) Microbial transformations in a cyclodextrin medium. Part 3. Cholesterol oxidation by *Rhodococcus erythropolis*. Appl. Microbiol. Biotechnol. 40: 230–240

Janosz-Rajczyk M (1991) Evaluation of the biodegradation of surfactants using IR and proton NMR spectrometry techniques. Vom Wasser 77: 21–34

Jing SR, Benefield LD & Hill WE (1992) Observations relating to enhanced phosphorus removal in biological systems. Water Res. 26: 213–223

Jun S, Chung KH & Moon CH (1996) Adsorption of cadmium species on montmorillonite investigated by ^{113}Cd NMR spectroscopy. Environ. Technol. 17: 655–660

Jurkiewicz A & Machiel GE (1995) Solid-state ^{13}C NMR studies of the interaction of acetone, carbon tetrachloride and trichloroethylene with soil components. Sci. Total Environ. 164: 195–202

Ke H-Y & Rayson GD (1990) Characterization of Cd binding sites on *Datura innoxia* using ^{113}Cd NMR spectroscopy. Environ. Sci. Technol. 26: 1202–1205

Keith LH & Hercules SH (1973) Environmental applications of advanced instrumental analyses. Assistance projects FY 69-71. U. S. Environ. Prot. Agency, Off. Res. Dev., [Rep.] EPA

Kentgens APM (1997) A practical guide to solid-state NMR of half-integer quadrupolar nuclei with some applications to disordered systems. Geoderma 80: 271–306

Kenyon WE (1992) Nuclear magnetic resonance as a petrophysical measurement. Nucl. Geophys. 6: 153–171

Kiwi J, Pulgarin C, Peringer P & Graetzel M (1993) Beneficial effects of homogeneous photo-Fenton pretreatment upon the biodegradation of anthraquinone sulfonate in waste water treatment. Appl. Catal. B 3: 85–99

Kleinberg RL (1994) Pore size distributions, pore coupling, and transverse relaxation spectra of porous rocks. Magn. Reson. Imag. 12: 271–274

Knicker H & Lüdemann H-D (1995) ^{15}N and ^{13}C CPMAS and solution NMR studies of ^{15}N-enriched plant material during 600 days of microbial degradation. Org. Geochem. 23: 329–341

Knicker H, Hatcher PG & Scaroni AW (1996) A solid-state ^{15}N NMR spectroscopic investigation of the origin of nitrogen structures in coal. Int. J. Coal Geol. 32: 255–278

Kögel-Knabner I (1997) ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80: 243–270

La Heij EJ, Kerkhof PJAM, Kopinga K & Pel L (1996) Determining porosity profiles during filtration and expression of sewage sludge by NMR imaging. AICHE J. 42: 953–959

Lens P, O'Flaherty V, Dijkema C, Stams A & Colleran E (1996) Propionate degradation by mesophilic anaerobic sludge: degradation pathways and effects of other volatile fatty acids. J. Ferment. Bioeng. 82: 387–391

Lens P, Hulshoff Pol L, Lettinga G & van As H (1997) Use of ^{1}H NMR to study transport processes in sulfidogenic granular sludge. *Wat. Sci. Technol.* 36: 157–163

Lens P, Dijkema C & Stams A (1998) ^{13}C-NMR study of propionate metabolism by sludges from bioreactors treating sulfate or sulfide rich wastewater. Biodegradation 9: 179–186

Lewandowski Z, Stoodley P, Altobelli S & Fukushima E (1994) Hydrodynamics and kinetics in biofilm systems – recent advances and new problems. Water Sci. Technol. 29: 223–229

Li Y, Neil J & Ackerman JJH (1995) On the use of ^{133}Cs as an NMR active probe of intracellular space *in vivo.* NMR Biomed. 8: 183–189

Lin KS, Wang HP & Kuo C-W (1996) Pyrolysis of auto shredder residue in hot motor oil. Proc. Int. Conf. Solid Waste Technol. Manage Paper 2C3

Lyngstad M & Grasdalen H (1993) A new NMR airlift bioreactor used in ^{31}P NMR studies of itaconic acid producing *Aspergillus terreus.* J. Biochem. Biophys. Methods 27: 105–116

Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H & Laufs R (1996) The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermis* is a linear ß-1,6-linked glucosaminoglycan: purification and structural analysis. J. Bacteriol. 178: 175–183

Mardon D, Prammer MG & Coates GR (1996) Characterization of light hydrocarbon reservoirs by gradient-NMR well logging. Magnetic Resonance Imaging 14: 769–777

Maroto-Valer MM, Andresen JM, Rocha JD & Snape CE (1996) Quantitative solid-state ^{13}C NMR measurements on cokes, chars and coal tar pitch fractions. Fuel 75: 1721–1726

Melvin BK & Shanks JV (1996) Influence of aeration on cytoplasmic pH of yeast in an NMR airlift bioreactor. Biotechnol. Prog. 12: 257–265

Mikame K, Watanabe T, Honda Y & Kuwahara M (1995) Structure and microbial decolorization of xylanase-resistant chromophoric xylans isolated from unbleached kraft pulp. Wood Res. 82: 28–30

Nagaoka T, Umezu K-I, Kouno K & Yoshida S (1996) Selective inhibitors of germination of legume seeds in activated sludge compost. Plant Growth Regul. 20: 295–302

Nanny MA, Bortiatynski JM, Tien M & Hatcher PG (1996) Investigations of enzymatic alterations of 2,4-dichlorophenol using ^{13}C NMR in combination with site-specific ^{13}C-labeling: understanding the environmental fate of this pollutant. Environ. Toxicol. Chem. 15: 1857–1864

Nestle N & Kimmich R (1996a) Heavy metal uptake of alginate gels studied by NMR microscopy. Colloids and Surfaces A: Physicochemical and Engineering Aspects 115: 141–147

Nestle N & Kimmich R (1996b) NMR microscopy of heavy metal absorption in calcium alginate beads. Appl. Biochem. Biotechnol. 56: 9–17

Nicholson JK & Wilson ID (1989) High resolution proton magnetic resonance spectroscopy of biological fluids. Progress in NMR Spectroscopy 21: 449–501

Ogoma Y, Kobayashi H, Fujii T, Kondo Y, Hachimori A, Shimizu T & Hatano M (1992) Binding study of metal ions to S100 protein: calcium-43, magnesium-25, zinc-65 and potassium-29 NMR. Int. J. Biol. Macromol. 14: 279–286

Omil F, Lens P, Hulshoff Pol L & Lettinga G (1997) Characterization of biomass from a sulphidogenic, volatile fatty acid-degrading granular sludge reactor. Enzyme Microbiol. Technol. 20: 229–236.

Oude Elferink SJWH, Lens PNL, Dijkema C & Stams AJM (1996) Isomerization of butyrate to isobutyrate by *Desulforhabdus amnigenus.* FEMS Microbiol. Lett. 142: 237–241

Pereira H, Lemos PC, Reis MAM, Crespo JPSG, Carrondo MJT & Santos H (1996) Model for carbon metabolism in biological phosphorus removal processes based on *in vivo* C-NMR labeling experiments. Water Res. 30: 2128–2138

Petroff PD (1975) Processed sewage effluent. US patent

Preston CM (1996) Applications of NMR to soil organic matter analysis: history and prospects. Soil Science 161: 144–166

Rasmussen S, Wolff C & Rudolph H (1996) 4′-O-β-D-glucosyl-cis-p-coumaric acid – a natural constituent of *Sphagnum fallax* cultivated in bioreactors. Phytochemistry 42: 81–87

Roeske I & Schoenborn C (1994) Influence of the addition of precipitants on the biological phosphorus elimination in a pilot plant. Water Sci. Technol. 30: 323–332

Rumpel C, Kögel-Knabner I, Becker-Heidmann P & Hüttl RF (1996) Multiple causes for elevated carbon content in recultivated mine soils in Lusatia, Germany. In: Botrell SH (Ed.) Proc. Fourth Int. Symp. Geochemistry of the Earth's Surface (pp. 461–6). Ilkley, Univ. of Leeds

Sato H, Eto S & Suzuki H (1980) Studies on the state of water in sludges by high-resolution NMR. Nippon Kagaku Kaishi 3: 354–358

Schmidt MWI, Knicker H, Hatcher PG & Kögel-Knabner I (1997) Impact of brown coal dust on the organic matter in particle-size fractions of a mollisol. Org. Geochem. 25: 29–39

Schoenborn C (1995) Characterization of P bonds in activated sludge. Schriftenr. WAR. 84: 1–16

Schuppenhauer MR, Kuhne G, Tiefenauer L, Smala A & Dunn IJ (1995) Non-invasive online investigations of industry style bioreactors. In: Breuvery EC et al. (Eds) Anim. Cell Technol.: Dev. 21st Century (pp. 865–869). Kluwer Academic Publishers, Dordrecht

Shin H-S & Lim K-H (1996) Spectroscopic and elemental investigation of microbial decomposition of aquatic fulvic acid in biological process of drinking water treatment. Biodegradation 7: 287–295

Sijbesma WFH, Almeida JS, Reis MAM & Santos H (1996) Uncoupling effect of nitrite during denitrification by *Pseudomonas fluorescens*: an *in vivo* ^{31}P NMR study. Biotech. Bioeng. 52: 176–182

Singh VP, Singh RV & Tandon JP (1990) Steriochemical and biochemical aspects of some organoboron complexes of sulfur donor ligands. J. Inorg. Biochem. 39: 237–246

Stilbs P (1987) Fourier transform pulsed-gradient spin-echo studies of molecular diffusion. Prog. Nucl. Magn. Reson. Spectrosc. 19: 1–45

Stöcker M (1996) Characterization of zeolitic materials by solid-state NMR – state of the art. In: Chon H, Woo SI & Park S-E (Eds) Recent advances and new horizons in zeolite science and technology, Vol 102 (pp 141–189). Elsevier, Amsterdam

Tallarek U, Albert K, Bayer E & Guiochon G (1996) Measurement of transverse and axial apparent dispersion coefficients in packed beds. AICHE Journal 42: 3041–3054

Tholozan JL, Samain E, Grivet JP, Moletta R, Dubourguier HC & Albagnac G (1988) Reductive carboxylation of propionate to butyrate in methanogenic ecosystems. Appl. Environ. Microbiol. 54: 441–445

Uhlmann D, Roeske I, Hupfer M & Ohms G (1990) A simple method to distinguish between polyphosphate and other phosphate fractions of activated sludge. Water Res. 24: 1355–1360

Van As H & van Dusschoten D (1997) NMR methods for imaging of transport processes in micro-porous systems. Geoderma 80: 389–403

Van Dusschoten D, Moonen CTW, de Jager PA & van As H (1996) Unravelling diffusion constants in biological tissue by combining CPMG imaging and pulsed field gradient NMR. Magn. Reson. Med. 36: 907–913

Vašák M (1998) Application of ^{113}Cd NMR to metallothioneins. Biodegradation 9: 501–512

Veeman WS (1997) Nuclear magnetic resonance, a simple introduction to the principles and applications. Geoderma 80: 225–242

Wais A, Haider K, Spiteller M, de Graaf AA, Burauel P & Fuhr F (1995) Using ^{13}C-NMR spectroscopy to evaluate the binding mechanism of bound pesticide residues in soils. J. Environ. Sci. Health B 30: 1–25

Young IR, Hand JW, Oatridge A & Prior MV (1994) Modeling and observation of temperature changes *in vivo* using MRI. Magnetic Resonance in Medicine 32: 358–369

Yoshida T & Kojima H (1978) Studies on environmental safety of di-isopropylnaphthalene (DIPN). Part II. Biodegradation of ^{14}C-DIPN with activated sludge. Chemosphere 7: 497–501

Zupke C & Foy B (1995) Nuclear magnetic resonance analysis of cell metabolism. Curr. Opin. Biotechnol. 6: 192–197