



USE OF ^1H NMR TO STUDY TRANSPORT PROCESSES IN SULFIDOGENIC GRANULAR SLUDGE

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ABSTRACT

Pulsed field gradient nuclear magnetic resonance (NMR) techniques have been applied to study diffusion and flow in a sulfidogenic granular sludge bed. When sulfidogenic granular sludge is exposed to a 20 MHz magnetic field, a multi-exponential spin-spin relaxation (T_2) with at least 5 populations is observed. One of these populations ($T_2 \approx 30$ ms) is intracellular water. Diffusion measurements at 22°C with ^1H -water as tracer indicated that sulfidogenic granular sludge contains a distribution of diffusion coefficients between 1.0×10^{-9} m²/s and 2.1×10^{-9} m²/s. Analysing the data set using a monoexponential fit gives a general parameter that can be used to describe the apparent diffusion coefficient in granular sludge. This approach showed that sulfidogenic granular sludge cultivated in different reactor configurations (UASB, USSB and baffled reactors) has comparable diffusional characteristics. Finally, the use of flow and imaging measurements in sulfidogenic granular sludge beds is discussed. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Sulfidogenic; granular sludge; NMR; relaxation; diffusion; flow.

INTRODUCTION

There is growing interest in operating Upflow Anaerobic Sludge Bed (UASB) reactors under sulfidogenic conditions for the treatment of sulfate rich wastewater. This new application of the UASB process, which treats wastewaters with a chemical oxygen demand (COD) to sulfate ratio lower than 1, allows combined COD and sulfate removal (Lettinga, 1995). In sulfidogenic reactors, wastewater purification is mainly accomplished by sulfate reducing bacteria, which convert sulfate to dissolved sulfide. As methanogenic bacteria are not involved in the degradation of organic matter, less gaseous end products (e.g. no methane gas) are formed. However, biogas evolution has been reported to reduce the external diffusion resistance of granular sludge (Huisman *et al.*, 1990), imposed by the surrounding stagnant liquid layer of some 200 μm (Lens *et al.*, 1993). Consequently, sulfidogenic reactors operate at lower mass transfer rates.

To improve the operation of sulfidogenic granular sludge reactors, more information about the substrate transport processes are required. This information can be provided by NMR, a non invasive technique which

requires no deactivation of the biocatalyst and allows *in vivo* and *in situ* measurements in the reactor. It thus overcomes the disadvantages of microelectrode measurements to characterise mass transfer phenomena, where measurements have to be done *ex situ* (in a flow cell) on selected, spherical and deactivated aggregates (Lens *et al.*, 1993).

Nuclear Magnetic Resonance (NMR) techniques using water as the tracer molecule have been developed to characterise different types of flow (diffusion, perfusion, turbulent flow) in plants, mushrooms, organs, soils and micro-porous (bio)systems (Schaafsma *et al.*, 1992). In this paper, we demonstrate the application of these NMR techniques to characterise transport processes in a bed of sulfidogenic granular sludge.

MATERIALS AND METHODS

Source of biomass

A sulfidogenic UASB reactor and upflow staged sludge bed (USSB) reactor, where the sludge bed was staged in five vertically orientated compartments, were operated for 140 days (Lens *et al.*, 1997a). A three stage baffled reactor was subsequently inoculated with sludge that had developed in the sulfidogenic UASB reactor and operated for 38 days (Bakker, 1996). All reactors were fed a volatile fatty acid mixture (acetate:propionate:butyrate in a ratio of 1:2:2 on COD basis; COD/sulfate ratio 0.5; pH 8.0; 30°C) at a specific organic loading rate of 0.5 g COD/g VSS.d. Sludges were sampled at the end of each experimental run.

NMR measurements

NMR measurements were done at room temperature ($22 \pm 1^\circ\text{C}$) on a 0.47 T (20.35 Mz) imager consisting of a Bruker electromagnet, a SMIS console and a DOTY probe head with actively shielded gradients and a 4.5 cm diameter cylindrical sample space (van Dusschoten *et al.*, 1995a). T_2 and diffusion measurements were performed with all sulfidogenic granular sludge types in a 2 cm inner diameter glass tube containing about 20 ml gravity settled sludge, rinsed with tap water. For T_2 and diffusion measurements, about 50 μl iron chelated dextran (size about 25 nm) was added to the sample to decrease the T_2 of the extra-granular water to about 1 ms. Flow measurements were done in a 20 cm long glass column (3 cm inner diameter). The column was packed with sulfidogenic granular sludge and flushed upwards with anaerobic tap water during flow measurements. All figures presented relate to the sludge developed in the first compartment of the baffled reactor.

Determination of T_2 . T_2 was measured using a modified Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Snaar and Van As, 1992). T_2 distributions were calculated using CONTIN analysing software.

Diffusion measurements. Diffusion coefficients were determined using diffusion analysis by relaxation time separated pulsed field gradient nuclear magnetic resonance (PFG NMR), as described by van Dusschoten *et al.* (1995a). Typical acquisition parameters were: spectral width 100 kHz, number of echoes and averages = 2000 and 4, respectively, $T_r = 6$ s, $\delta = 5$ ms and $\Delta = 12.1$ ms. Data were analysed using CONTIN or non linear least square (NLLS) fitting routines (van Dusschoten *et al.*, 1995a).

Flow measurements. Flow measurements were determined using the PFG stimulated echo sequence and data were analysed using NLLS fitting routines as described by van Dusschoten *et al.* (1995b). Typical acquisition parameters were: 64 gradient steps, $G_{\text{max}} = 13068$ Hz/mm, number of averages = 2, $T_r = 3$ s, $\delta = 4$ ms and $\Delta = 932$ ms.

RESULTS AND DISCUSSION

T₂ Relaxometry

The ¹H NMR spin-spin relaxation time T₂ is an important parameter used in the characterisation of microporous materials (Schaafsma *et al.*, 1992). This parameter expresses the magnetisation decay rate after exposing a sample to a magnetic field. Fig. 1A shows a typical magnetisation decay curve after exposing sulfidogenic granular sludge to a magnetic field of 20 MHz. Such curves follow the evolution in time of the amplitude of the top of CPMG echoes, generated at an inter-pulse time, 2τ, of 0.49 ms.

The ¹H (T₂) relaxation in pure water has a typical value of 2.0 to 2.5 s. Generally, ¹H relaxation of water in microporous systems is multiexponential and much faster than that of bulk water. Figure 1B shows indeed that 6 distinct water ¹H populations can be distinguished in the T₂ decay curve of sulfidogenic granular sludge (Fig. 1A), with T₂ values considerably shorter than that of pure water. The decrease in T₂ in biologically active micro-porous systems is due to exchange of protons with soluble cellular or intercellular molecules, heterogeneity in cell and pore distribution, subcellular compartments and relaxation sinks at the boundaries of homogeneous compartments. Similar broad distributions of T₂ values have been observed in porous rocks (Kleinberg, 1994), apple parenchyma (Snaar and Van As, 1992), immobilised cell beads (Beuling *et al.*, 1997) and mesophilic methanogenic aggregates (Lens *et al.*, 1997b).

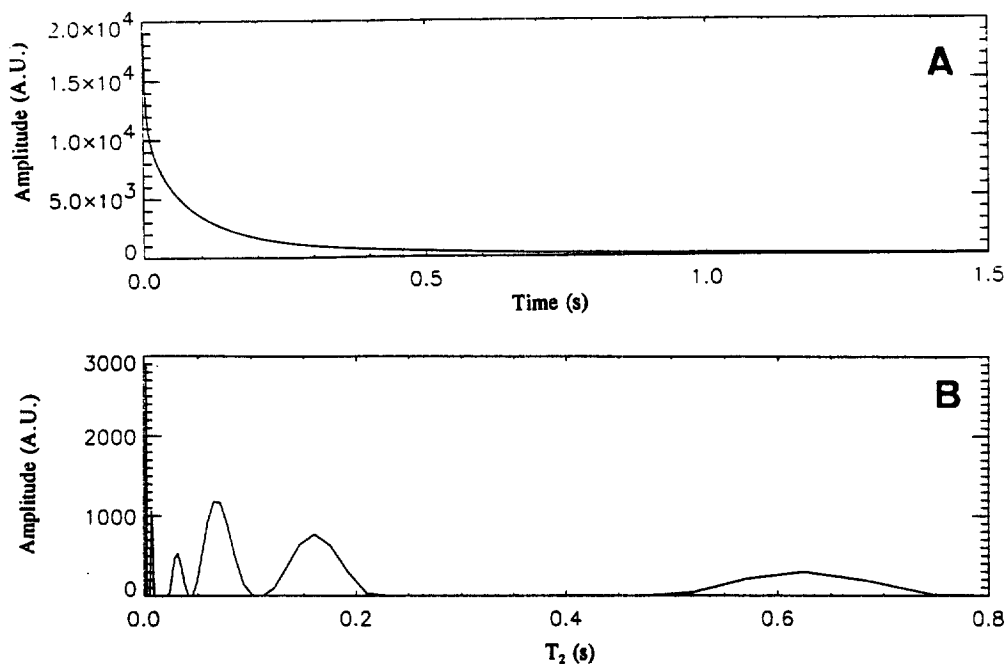


Figure 1. (A) Typical multi-exponential T₂ decay curve of the magnetisation as a function of time.
(B) T₂ populations extracted from this multi-exponential T₂ decay curve using CONTIN.

Multi-exponential T₂ decay has been suggested to reflect water present in different anatomic compartments of a system. The pore size distribution of porous rocks can be extracted from the T₂ distribution (Kleinberg, 1994). In apple tissues, three water populations could be observed in the T₂ distribution, which corresponded with the vacuole, the cytoplasm, and the cell wall/extracellular space (Snaar and Van As, 1992). With respect to the measurements performed in sulfidogenic granular sludge (Fig. 1B), the ¹H population with a T₂ of about 1 ms is extra granular water containing paramagnetic iron chelated to dextran used in the

diffusion measurements (see below). Relaxation measurements in methanogenic granular sludge assigned the ^1H population with a T_2 of about 30 ms to bacterial intracellular water, whereas the ^1H populations with the longer T_2 values (625 ms and 160 ms) most probably reflect water present in cavities or gas evolution channels (Lens *et al.*, 1997b).

Diffusion measurements

Being sensitive to molecular displacements in the micrometre range, PFG NMR proved to be a versatile tool for studying mass transfer phenomena in heterogeneous systems. PFG NMR procedures determine the average displacement of an ensemble of molecules over a specified time Δ (van Dusschoten *et al.*, 1995a). Its popularity arises from the fact that the observed signal attenuation is proportional to the Einstein diffusion constant (D) and three experimental adjustable NMR parameters: the strength (G), duration (δ) and interval (Δ) of the magnetic field gradient pulses. The natural logarithm of the signal attenuation versus $\beta^2 G^2 \delta^2 (\Delta - \delta/3)$, the b factor, results in a signal attenuation plot. The slope of this plot represents the D of the sample.

For sulfidogenic granular sludge that contains different water ensembles, as shown by the T_2 distribution (Fig. 1), PFG NMR measurements will give a diffusion coefficient for each of these ensembles, as the echo amplitude $S(t,b)$ is proportional to:

$$S(t,b) \approx \sum S_{0,n} \exp(-TE/T_{2,n}) \exp(-bD_n)$$

with n the number of distinguishable water ensembles and β the gyromagnetic ratio.

The diffusion analysis by relaxation time separated PFG NMR applied in this study relates the diffusion coefficients to their corresponding T_2 . This procedure records T_2 decay curves at increasing magnetic field gradients (Fig. 2A). Thus apparent diffusion coefficients can be determined from the y-axis of Fig. 2A, after extracting the T_2 distribution from the x-axis. It should be noticed that in Fig. 2B, there are no data any more about the extra granular water with a typical T_2 of about 1 ms. This is because this water ensemble relaxes during the magnetisation preparation period Δ and thus cannot contribute to the measured signal amplitude any more.

Table 1. Mono and bi-exponential analysis of D_{app} (mean of triplicate measurements) determined by PFG NMR in various types of sulfidogenic granular sludge. Corresponding diffusion coefficient of free water = $2.20 \times 10^{-9} \text{ m}^2/\text{s}$.

Sludge type*	Monoexponential		Biexponential			
	T_2 (ms)	D_{app} ($10^{-9} \text{ m}^2/\text{s}$)	T_{21} (ms)	D_{app1} ($10^{-9} \text{ m}^2/\text{s}$)	T_{22} (ms)	D_{app2} ($10^{-9} \text{ m}^2/\text{s}$)
Inoculum	91	1.60	55	1.37	294	2.19
UASB	153	1.57	78	1.32	410	1.98
USSB						
Comp 1	260	1.70	102	1.52	515	1.83
Comp 2	161	1.61	92	1.47	303	1.77
Comp 3	153	1.59	87	1.37	400	1.90
Comp 4	163	1.48	68	1.37	357	1.57
Comp 5	167	1.67	92	1.47	333	1.92
Baffled						
Comp 1	119	1.48	76	1.30	427	2.04
Comp 2	143	1.45	89	1.27	549	2.01
Comp 3	127	1.48	84	1.28	400	2.06

* Comp: Compartment; Comp 1 refers to the compartment with the influent supply

Figure 2B shows that sulfidogenic granular sludge does not contain one single apparent diffusion coefficient (D_{app}), but actually consists of a distribution of diffusion coefficients. This D_{app} distribution corresponds to a distribution of T_2 values and might be related to density gradients in biofilms (Fu *et al.*, 1994). The information given in Fig. 2B is, on the other hand, too detailed for commonly used mathematical models, where usually one value of D_{app} is used as entry. Therefore, mono- and bi-exponential analysis of the diffusion data set can be applied (Table 1). The mono-exponential analysis can be considered as a general D_{app} of the sludge. Table 1 further shows that a biexponential fit of the data set gives some more detailed information, although neither fraction can be used to compare granules in more detail.

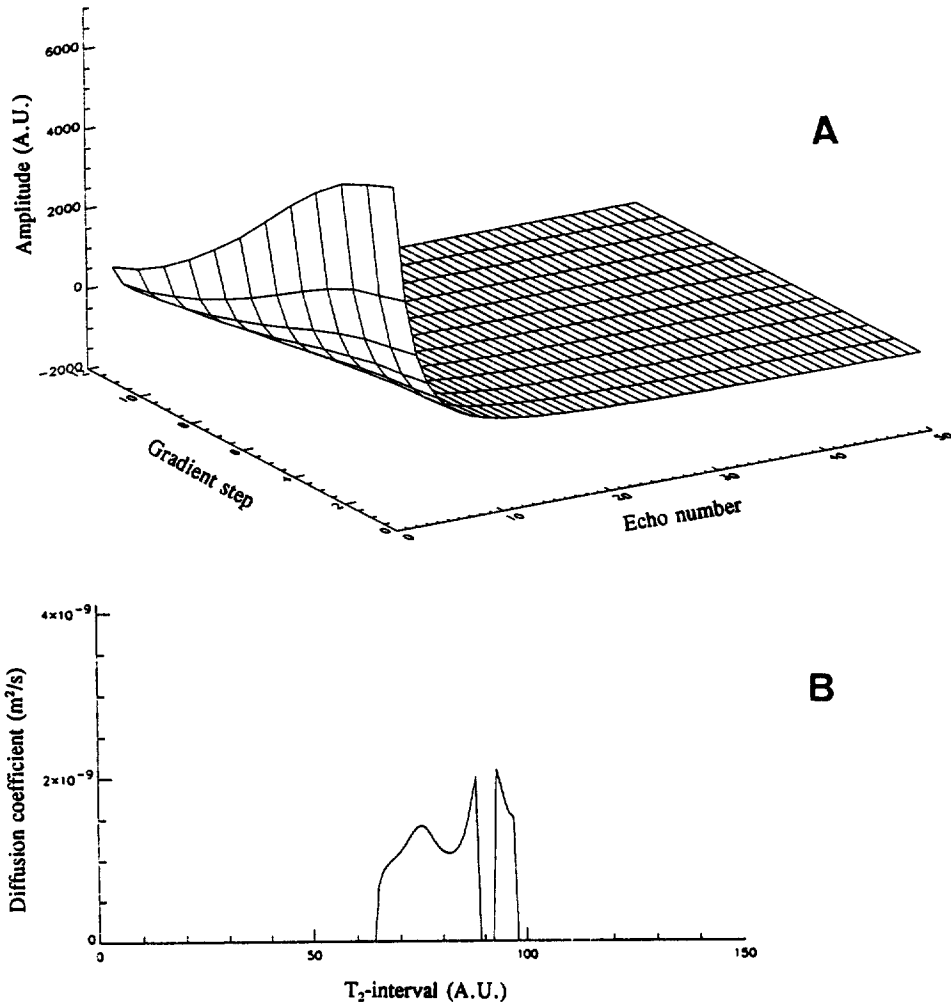


Figure 2. (A) Typical data set obtained by the PFG NMR diffusion measurements in sulfidogenic granular sludge. (B) Distribution of the diffusion coefficient as a function of T_2 intervals.

NMR is a non invasive method that allows *in vivo* measurements and thus is an elegant method to further investigate the effect of substrate and hydraulic loading rate, feed composition or operation temperature on the D_{app} . As an example, Table 1 shows that sulfidogenic granular sludges developed in the different bioreactor types had comparable diffusional properties. Also the effects of toxicants, i.e. those commonly used in transient state diffusion coefficient measurements (e.g. $HgCl_2$ or glutaraldehyde), on the D_{app} can be easily evaluated by this NMR procedure. NMR also allows us to measure T_2 and diffusion coefficients

spatially resolved by measuring these parameters for each volume element (voxel) after dividing the probe in a (128 x 128) grid. Recent adaptations of both the pulse sequence and the probe enable us to image T_2 and D_{app} of granular sludges with an in-plane resolution of $20\ \mu\text{m} \times 20\ \mu\text{m}$ for a slice of 1 mm (data not shown).

The presented diffusion data relate to ^1H of water. Its diffusivity obtained by ^1H NMR after a mono-exponential fit is comparable to the diffusivity of glucose, as measured with glucose microelectrodes (Beuling *et al.*, 1997). NMR procedures are, however, not restricted to ^1H of water. The D_{app} of the ^1H of specific organic molecules, i.e. glucose or volatile fatty acids can be determined using chemical shift imaging or double quantum editing techniques. These techniques depress the signal of the extremely abundant water molecules (about 55 M) in order to visualise protons of organic compounds usually present in the mM or μM range. Alternatively, other nuclei can be used as tracer, as for example the use of ^{19}F NMR to probe directly the D_{app} of fluorated organic molecules (e.g. ^{19}F -glucose).

Flow measurements

The maximum information about molecular mass transfer in heterogeneous systems accessible by PFG NMR is contained in the mean propagator (Kärger and Pfeifer, 1994), which is defined as the mean of the conditional probability density of molecular displacement over all starting positions. The average propagator can be determined using PFG stimulated echo (PFG-STE) NMR (van Dusschoten *et al.*, 1995b).

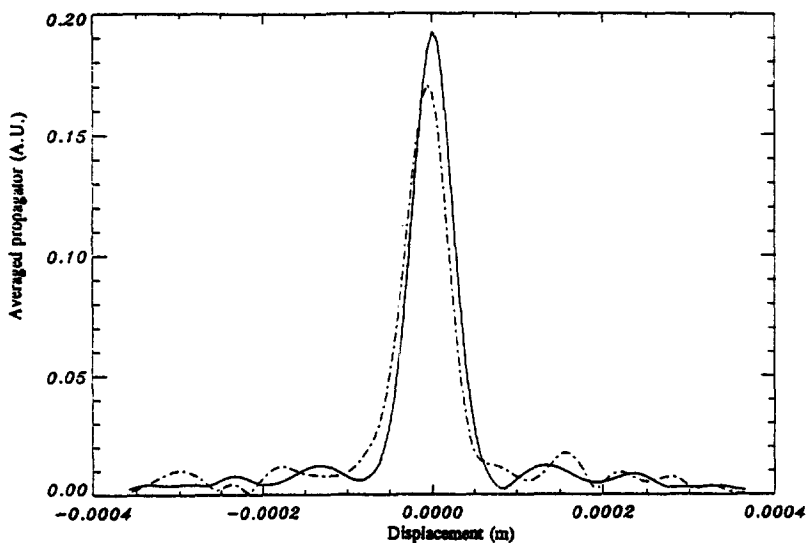


Figure 3. Effect of flow on the averaged propagator in a sulfidogenic granular sludge bed. (---) No flow; (—) Flow velocity of 16 ml/min.

Figure 3 shows the effect of the flow velocity on the propagator in a sludge bed of sulfidogenic granular sludge. For random motion (diffusion or dispersion; no flow), the correlation between the initial position r_0 and r is completely lost and the propagator is Gaussian. For net flow of spins, there is a distinct difference between r_0 and r . Consequently, the propagator is not symmetric around the origin. In the granular sludge bed presented in Fig. 3, the dispersion coefficient increased by 6% when the flow rate was increased from 0 to 16 ml/min. In case of flow, the propagator contains also information about the average flow velocity. Thus, the presence and role of convective transport in granular sludge (Kato *et al.*, 1994) can be evaluated using this NMR procedure. Using PFG-STE NMR imaging, localised flow velocity profiles can be measured within the granular sludge bed. Thus, the hydrodynamics of a granular sludge bed can be described by NMR and applied in hydrodynamic models.

CONCLUSIONS

PFG NMR spectroscopy measurements at low magnetic field strengths (20 MHz) allow us to depict both diffusive and flow transport processes in sulfidogenic granular sludge beds.

T_2 decay in sulfidogenic granular sludge discriminates five different water populations. One of these is intracellular water. Further research is required to uniquely relate the other water populations to the granular sludge anatomy.

Sulfidogenic granular sludge contains a distribution of diffusion coefficients.

Reactor operation for 178 days and staging of the sludge bed do not significantly alter the diffusional characteristics of sulfidogenic granular sludge.

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