Adsorption of vitamin B_{12} to alumina, kaolinite, sand and sandy soil

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Abstract

Vitamin B_{12} is an important component of media used to stimulate the growth of many anaerobic microbes that dechlorinate aliphatic compounds. Its delivery to the subsurface is therefore a concern for enhanced in situ bioremediation and bioaugmentation. In situ circulation of vitamin B_{12} and a chemical reductant has also been field tested for treatment of chlorinated methanes, ethanes, and ethenes. The objective of this study was to determine the extent of B_{12} (added as cyanocobalamin) adsorption to aquifer solids. Batch studies indicated a minor amount of adsorption to kaolinite clay (Freundlich $K_f = 1.5 \ (\mu g/g)(mL/\mu g)^{1/n}$ and $1/n = 0.39$) and sand ($K_f = 0.70 \ (\mu g/g)(mL/\mu g)^{1/n}$ and $1/n = 0.84$), and no detectable adsorption to alumina. Column studies using a ${}^3H_2O$ tracer and vitamin B_{12} in distilled water ($10 \ \mu M$) resulted in a B_{12} retardation factor (R) of approximately 2 for both sand (0.14% organic carbon) and a sandy soil (2.5% organic carbon). Using groundwater containing organic carbon (2.8 mg/L) in place of distilled water did not significantly change R. The relatively low value for R indicates vitamin B_{12} can be distributed throughout a contaminated aquifer without experiencing significant losses to adsorption.

1. Introduction

The importance of vitamin B_{12} to microbial metabolism is well-established. B_{12} is a common component of media for cultivating anaerobes. It is typically added in nanomolar to micromolar quantities along with other growth factors such as biotin and folic acid [1,2]. In addition, B_{12} has received considerable attention for its role as a transition metal coenzyme in transformation of numerous halogenated aliphatic compounds, including carbon tetrachloride [3,4], chloroform [5], chlorofluor-ocarbons [6], and chlorinated ethenes [7]. B_{12} is a required growth factor for *Dehalococcoides ethenogenes*,

the only isolate obtained thus far that can completely reduce tetrachloroethene to ethene via halorespiration [8]. Addition of B_{12} to an enrichment culture containing *D. ethenogenes* was required to sustain reductive dechlorination of tetrachloroethene over a period of several years when butyric acid was provided as the electron donor [9]. Cyanocobalamin (CN-Cbl) is the most economical form of vitamin B_{12} in common use.

As interest in enhanced bioremediation and bioaugmentation continues to develop for in situ treatment of chlorinated aliphatic compounds, there is a need to understand the factors affecting subsurface delivery of growth substrates and growth factors, including B_{12}. For example, B_{12} was a component in the medium used to develop the TCE dechlorinating "Pinellas culture," which was then pumped into an aquifer at Dover Air

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Force Base to evaluate the effectiveness of bioaugmentation [10]. Addition of B_{12} to the subsurface to enhance the rate of carbon tetrachloride transformation under acetogenic conditions, as well as to minimize the extent of conversion to chloroform, has been proposed [4]. In situ treatment of carbon tetrachloride and 1,2,2,2-tetrachloroethene by injecting a mixture of B_{12}, titanium citrate, and glucose has been field tested in a recirculating well at Aberdeen Proving Grounds, with promising results [11].

One concern with subsurface delivery of nutrients is the magnitude of adsorption, since this can significantly impact the amount that needs to be injected. Very little information is available on CN-Cbl adsorption to aquifer solids. The high solubility of CN-Cbl in distilled water (120 g/L) indicates a low potential for adsorption. Conversely, CN-Cbl is a large compound (MW = 1355.4) with numerous ionic groups, suggesting the potential for some degree of partitioning to solid surfaces. Kliewer and Morra [12] demonstrated that adsorption of dicyanocobinamide and aquocobalamin consistently exceeded adsorption of CN-Cbl onto Ca^{2+}-, K^+-, and Na^+-hectorite, based on batch tests with millimolar levels of the corrinoids. The objective of our study was to determine the extent to which micromolar concentrations of CN-Cbl adsorb to alumina, kaolinite, sand, and sandy soil, using batch and column experiments.

2. Methods and materials

2.1. Chemicals and analytical procedures

CN-Cbl (99%), alumina, and kaolinite clay were obtained from Sigma, ^3H_2O (0.58×10^6 disintegrations/min/mL) from the Radioisotope Laboratory of the University of Illinois at Urbana. EcoScint (Baker Diagnostics, Inc.) was used as the liquid scintillation cocktail for ^3H quantification.

CN-Cbl was analyzed using a 250-mm × 4.6-mm RP–318 column (Bio–Rad Laboratories) held at 30°C and a Hewlett-Packard 1090 Series II high-performance liquid chromatograph equipped with an autosampler and a diode array detector. The mobile phase consisted of 30% acetonitrile in 1 mM ammonium acetate buffer, at a flow rate of 1.4 mL/min (modified from [13]), resulting in an elution time of approximately 14 min. Samples were filtered (0.22 μm pore size) prior to injection (100 μL). The detection limit for CN-Cbl was 24 nM at a wavelength of 254 nm.

2.2. Batch adsorption tests

Batch adsorption tests were conducted at ambient temperature (21 ± 2°C) with sand (bulk density = 1.63 g/cm³, porosity = 0.36, organic carbon content = 0.14%, and nominal diameter = 0.25–0.35 mm), alumina, and kaolinite (bulk density = 0.66 g/cm³, porosity = 0.75) using the bottle point technique [14]. Varying concentrations of CN-Cbl dissolved in distilled water were added to 160-mL serum bottles, yielding an adsorbent to solution ratio between 1:4 and 1:20. The mixture was equilibrated for one week on a rotary shaker and then analyzed for CN-Cbl. Equilibrium concentrations ranged from 0.6 to 5.2 μM. Data were fit to the two-parameter nonlinear Freundlich isotherm model for a single solute:

$$q_e = K_f C_e^{1/n}, \qquad (1)$$

where q_e is the equilibrium concentration of solute in the sorbed phase (μg/g), K_f is the sorption coefficient, related to the capacity of the sorbent ((μg/g)(L/mg)$^{1/n}$), C_e is the equilibrium concentration of the solute (mg/L), and $1/n$ reflects the degree of nonlinearity for the isotherm. The concentration of solute in the sorbed phase was determined by

$$q_e = \frac{[(C_0 - C_e)V - \beta C_e]}{M}, \qquad (2)$$

where C_0 is the initial concentration of the solute (mg/L), V is the volume of solution (L), M is the mass of soil (g), and β is an adjustment factor for the mass adsorbed to the bottle surface and septum (no soil present).

2.3. Column adsorption tests

Column adsorption experiments were conducted in a 450-mm × 51-mm liquid chromatography glass column (Ace Glass, Inc.) with a total void volume of 240 mL (Fig. 1). The ends were fitted with Teflon screw caps and stainless steel reducers. It was packed with approximately 850 g of sand (same as described above) or a sandy soil (bulk density = 1.37 g/cm³, porosity = 0.39, organic carbon content = 2.49%, sand 52.5%, silt 28.1%, clay 19.4%, cation exchange capacity = 21 meq/100 g, and pH = 7.31). The columns were prepared as previously described [15], with sand or sandy soil sandwiched between a layer of glass wool and glass beads (0.5-mm) at the inlet and outlet. Before the test period with CN-Cbl, at least 30 pore volumes of 0.01 M $CaCl_2$ solution were pumped through using a high-performance liquid chromatography pump (Model 501, Waters, Inc.). The experiment was started by pumping a solution containing CN-Cbl (10 μM) and ^3H_2O (675 disintegrations/min/mL) at a flow rate of 0.2 mL/min (pore water velocity = 0.14 m/day). The effluent was collected in 20–50 min intervals with a fraction collector (Waters, Inc.), filtered (0.22 μm), and analyzed for CN-Cbl and ^3H activity.
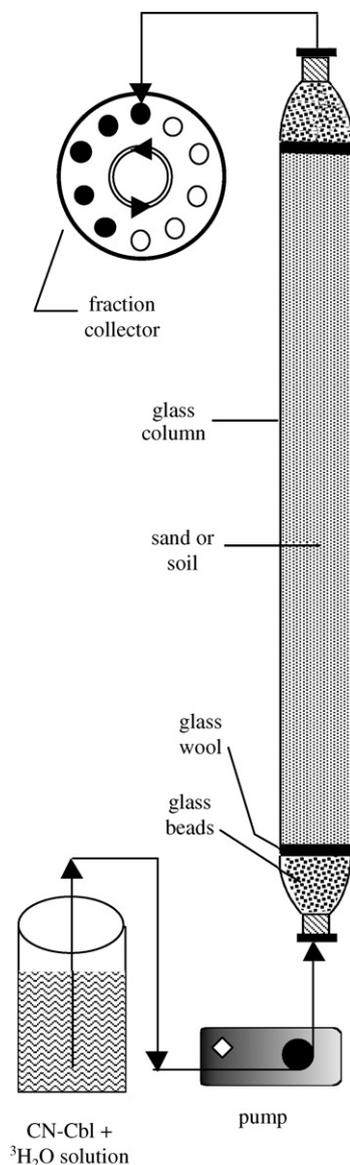
Fig. 1. Schematic of the column system used to measure adsorption of CN-Cbl to sand and sandy soil.

3. Results and discussion

The Freundlich isotherm parameters K_f and $1/n$ for CN-Cbl were obtained by fitting the linearized version of Eq. (1). For sand, $K_f = 0.70$ (μg/g) (mL/μg)$^{1/n}$ and $1/n = 0.84$ with a coefficient of determination (R^2) of 0.88, based on 12 data points (2 each at three initial concentrations and 6 points at one initial concentration). For kaolinite, $K_f = 1.5$ (μg/g) (mL/μg)$^{1/n}$ and $1/n = 0.39$ with a R^2 of 0.90, based on 10 data points (2 points each at two initial concentrations and 6 at one initial concentration). For alumina, so little adsorption

occurred that it was not possible to determine isotherm parameters.

Although the R^2 values were reasonable (0.88 and 0.90), the distribution of residuals for one initial concentration employing 6 data points was non-random, suggesting that the Freundlich model may not be fully describing the adsorption behavior of CN-Cbl. A similar non-random pattern in residuals for the Freundlich model was reported by Kliewer and Morra [12]. They presented CN-Cbl isotherms for three types of hectorite but did not report any model parameters for adsorption. Also, Kliewer and Morra [12] used CN-Cbl concentrations as high as 830 μM, while the highest concentration used in this study was only 5.2 μM, making direct comparisons questionable. No other studies were found that evaluated batch adsorption of CN-Cbl.

The retardation factor (R) is the ratio of groundwater velocity to solute velocity, which is directly related to the extent of solute adsorption. R can be predicted as follows:

$$R = 1 + \frac{\rho_B}{\varepsilon} k_d, \tag{3}$$

where ρ_B is the bulk density of the sorbent (g/mL), ε is the effective porosity, and K_d is the soil partition coefficient (mL/g). K_d can be estimated from the linear portion of the Freundlich isotherm, at low concentrations (where n is close to 1). For sand and CN-Cbl in distilled water, the linear portion applies up to approximately 9 μg CN-Cbl/mL (6.6 μM), and in this region K_d is 0.50 mL/g. Using Eq. (3), the predicted R for CN-Cbl in sand is 3.3. Since the isotherm for kaolinite is linear at only very low concentrations (<1 μg/mL), it is not appropriate to use the isotherm parameters to predict R for this sorbent.

Adsorption of CN-Cbl to the same type of sand used in the batch tests was evaluated in column tests, using both distilled water and a sample of groundwater (pH = 7.2, Ca^{2+} = 54 mg/L, Mg^{2+} = 28 mg/L, alkalinity = 276 mg/L as CaCO$_3$, total organic carbon = 2.8 mg/L). Breakthrough curves for ^{3}H$_2$O and CN-Cbl are shown in Fig. 2a. The R for CN-Cbl in distilled water is 1.9, calculated by dividing the area projected on the y-axis from the breakthrough curve for CN-Cbl to the area projected for the ^{3}H$_2$O curve. This is reasonably close to the R predicted above using the batch data and Eq. (3). The R for CN-Cbl in groundwater is 2.1, indicating no substantial affect of the dissolved organic matter versus using distilled water.

Adsorption of CN-Cbl to a higher organic content sandy soil (2.49%) was evaluated in another column test, using only distilled water (Fig. 2b). The breakthrough pattern for CN-Cbl was similar to the lower organic carbon sand, resulting in a slightly higher R of 2.4. Using Eq. (3) and R from the column test, the
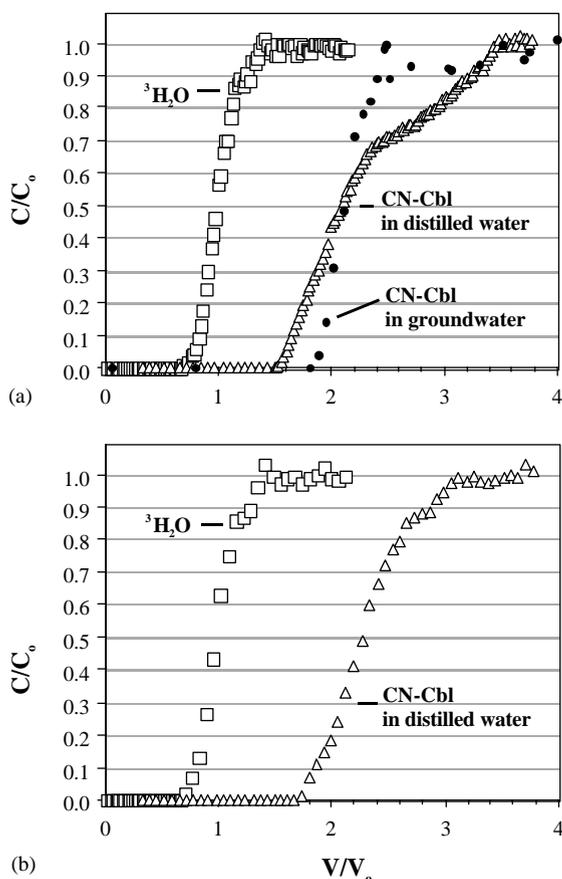
Fig. 2. Breakthrough curves for tritium and CN-Cbl in distilled water and groundwater passing through a low organic carbon sand (a); and CN-Cbl in distilled water passing through a higher organic carbon sandy soil (b). C = [CN-Cbl], C_0 = initial [CN-Cbl] (10 μM); V = volume of water collected; V_o = pore water volume (empty bed volume × porosity).

calculated value of K_d for the sandy soil is 0.40 mL/g, similar to the value for the lower organic content sand (0.50 mL/g).

A mass balance on CN-Cbl for the column experiment with sand and distilled water (Fig. 2a) indicates that a total of 11.35 mg of CN-Cbl was added (0.837 L × 0.010 mmol/L × 1355.4 mg/mmol), of which 64.2% (7.29 mg) was recovered in effluent samples plus pore water remaining in the column. The difference, 35.8% (4.06 mg), was presumably adsorbed to the sand. Using Eq. (1) and the Freundlich parameters for sand, the estimated adsorption capacity at equilibrium with 10 μM (13.6 mg/L) is 6.25 μg CN-Cbl/g sand. The column contained 850 g of sand, so the predicted total amount of CN-Cbl adsorbed is 5.31 mg. This is about 30% higher than the observed amount, consistent with previous observations of potential inconsistencies between batch isotherm data and results from flow-

through columns [16]. The mass balance for tritium recovered in effluent samples plus pore water was 100.4%.

For the column experiment with sand and groundwater (Fig. 2a), the mass balance indicated 32.9% of the total CN-Cbl added was adsorbed. For the experiment with sandy soil and distilled water (Fig. 2b), the mass balance indicated 37.4% of the total CN-Cbl added was adsorbed. Thus, use of groundwater versus distilled water or sandy soil versus sand had a relatively small impact on the magnitude of CN-Cbl adsorption.

Using the K_d values and the organic carbon content of the soils tested (0.14% for sand, 2.49% for sandy soil), organic carbon partitioning coefficients (K_{oc}) were calculated as 357 mL/g for sand and 16 mL/g for sandy soil. The similarity in K_d values between sand and sandy soil versus the substantial difference in K_{oc} values suggests that organic carbon is not the primary sorbent for CN-Cbl, at least with these types of materials. There often is not a correlation between sorption and organic carbon content with soils containing a low amount of organic carbon, such as the sand used in this study [17].

No other studies were found that determined R for CN-Cbl using column tests. Nevertheless, some information is available on the movement of large molecules in aquifer materials. Studies conducted with DNA [18], dyes and proteins [19], and oligomers [20] indicate that the pH, ionic strength, and mineralogy (e.g., clay content) of soils, rather than the organic carbon content, play a more important role in determining the extent of adsorption of large molecules. Values of R as high as 14 have been reported for DNA in fine sand containing 0.6% organic carbon [18]. The R of 2.4 for CN-Cbl determined for sandy soil in this study is relatively low and in the same range reported for carbon tetrachloride [21].

CN-Cbl is a neutral molecule; the +3 charge on the cobalt center is balanced by negative charges on the ring and the cyanide [12]. At pH values significantly above or below neutral, it is possible that ionization of CN-Cbl would affect the extent of its adsorption. We did not evaluate this potential impact, although the column tests did show that R was similar for CN-Cbl in distilled water and groundwater (pH = 7.2). Most applications of CN-Cbl will likely involve addition to groundwater with a pH conducive to biological activity (i.e., approximately 6–8), in which case the impact of pH on CN-Cbl adsorption is likely to be small.

Our batch and column studies were performed with CN-Cbl that contains Co(II). If added to an aquifer with a low redox potential (e.g., resulting from biological activity and/or addition of a chemical reductant), it is likely that the cobalt would be reduced to Co(I). The effect of cobalt oxidation state on adsorption of CN-Cbl is not yet known with certainty. However, results from Kliewer and Morra [12] suggest it would be minimal.

They showed that the corrin ring maintains redox activity even when the corrinoid is sorbed to Ca^{2+}-hectorite. Reduction of Co(III) to Co(I) followed by reoxidation to Co(III) did not alter the extent of corrinoid adsorption, suggesting that the cobalt center is not a significant factor in adsorption, regardless of its oxidation state.

In summary, batch and column adsorption tests indicate that CN-Cbl binds weakly to alumina, kaolinite clay, sand and sandy soil, resulting in a relatively low retardation factor of approximately 2. This suggests that it is feasible to effectively deliver CN-Cbl throughout a contaminated aquifer, where it can be used to stimulate halorespiration or enhance chemically mediated reductive dechlorination of methanes, ethanes, and ethenes.

Acknowledgements

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