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Enzymatic consumption of carbonyl sulfide (COS) by marine algae

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Abstract. We show that the marine algae *Mantoniella squamata*, *Prymnesium parvum*, and *Amphidinium klebsii* take up carbonyl sulfide (COS) from their surrounding medium. Inhibitor studies confirm that this COS uptake is catalyzed by the enzyme carbonic anhydrase, which was not detectable with conventional methods. As shown for *M. squamata*, the COS uptake can be dependent on the growth conditions. Furthermore, COS uptake shows a clear positive correlation with the COS concentration in the growth medium. The value of $K_{1/2}$ for the COS uptake was estimated to be around 222 mol/m^3. The COS consumption by the marine algae species investigated was estimated to be negligible compared to the photoproduction and hydrolysis of COS in seawater.

Introduction

COS (carbonyl sulfide) is a climatically relevant trace gas with an atmospheric concentration of about 500 ppt (0.5 pmol mol^{-1}; Torres et al. 1980; Rasmussen et al. 1982b; Khalil 1992). Due to its stability, COS reaches the stratosphere and sustains the stratospheric aerosol layer (Crutzen 1976). This aerosol layer is involved in the backscattering of incoming solar radiation. Hence, COS may contribute significantly to the earth's climate. Existing data for sources and sinks of COS remain insufficient for an exact estimation of global budgets (for a review see Chin & Davis 1993). Although the sources are estimated to be larger than the sinks, no clear increase in tropospheric COS concentration has been measured (Bingemer et al. 1990; Bandy et al. 1992; Rinsland et al. 1992), indicating that either the sources of COS have

been overestimated or that there are still unidentified sinks (Kesselmeier et al. 1997; Andreae & Crutzen 1997).

The largest sink for COS is vegetation (Chin & Davis 1993). It is well established that COS is assimilated by higher plants (Taylor et al. 1983; Brown & Bell 1986; Brown et al. 1986; Goldan et al. 1987, 1988; Fall et al. 1988; Kesselmeier et al. 1991, 1992; Bartell et al. 1993; Kesselmeier & Merk 1993), freshwater algae (Protoschill-Krebs et al. 1995), and cyanobacteria (Miller et al. 1989; Badger & Price 1990), probably by a process analogous to CO_2 uptake with carbonic anhydrase (CA) as the key enzyme (Kesselmeier et al. 1991; Protoschill-Krebs & Kesselmeier 1992; Protoschill-Krebs et al. 1995, 1996) hydrolysing COS to CO_2 and H_2S. Since CA is an ubiquitous enzyme, all organisms which exchange CO_2 by the support of CA should also be able to consume COS. Until now, no experiments on COS uptake by marine algae have been described. The purpose of this work was therefore to investigate COS consumption by carbonic anhydrase in various marine algal species.

Materials and methods

Algal species

The algae *Mantoniella squamata* (*Prasinophyceae*; Plymouth, Nr. LB, 1965/5), *Prymnesium parvum* (*Prymnesiophyceae*; Göttingen, Nr. B 125.79), and *Amphidinium klebsii* (*Dinophyceae*; Göttingen, Nr. B 36.80) were used in the experiments for the following reasons: *M. squamata* is one of the most primitive of the green alga (Melkonian 1990), making it an ideal organism for comparison of COS exchange data obtained from it with that from the freshwater species *Chlamydomonas reinhardtii* (Protoschill-Krebs et al. 1995). *P. parvum* (together with *P. patelliferum*) shows large bloom periods and therefore can become dominant (and toxic to fish) in parts of the ocean (Padilla 1970; Shilo 1971; Larsen et al. 1993). *A. klebsii* is one of the most important producers of organic compounds in the oceans (Van den Hoek & Jahns 1984; Van den Hoek et al. 1995).

Growth conditions for the marine algal species

All algal species were grown in semi-continuous cultures at 17 °C under a light/dark cycle (16 h/8 h) with a light intensity of 10 μmol photons m^{-2} s^{-1} in Erlenmeyer flasks (batch cultures). *M. squamata* was also grown in thermostat cultures (Kniese, Marburg, Germany) under continuous light (100 μmol photons m^{-2} s^{-1}), with the culture tubes being continuously

aerated with synthetic air. Growth was found optimal under these conditions for both culture regimes. Algal samples were taken from all cultures in the exponential growth phase. *A. klebsii* was grown in artificial seawater according to Schlösser (1982), whereas *M. squamata* und *P. parvum* were grown in artificial seawater according to Müller (1962). The pH of both growth media was 7.8. The medium according to Müller contained 1890 μg $ZnCl_2$ l^{-1}, whereas the medium according to Schlösser contained natural seasalt (reflecting the natural concentrations of trace elements in seawater) plus an additional unspecific soil extract with a zinc content of approximately 150 ppm (Jenkins & Jones 1980), resulting in a final zinc concentration of >2 mg Zn l^{-1}. Free zinc concentrations are not known for both media, because of binding to organic ligands (as in nature). However, the optimal growth rates obtained show that it should have been at least sufficient and furthermore well correlated to other essential ions. For more details concerning the growth of the algae see Wilhelm et al. (1986).

Algal assays

The algal assays followed primarily the method described by Protoschill-Krebs et al. (1996) for the isolated enzyme carbonic anhydrase. Aliquots of cell culture samples were pipetted into 1.85 cm^3 ampules, which were then sealed with gas-tight septa. The enzyme reaction with COS was started by injecting a buffered COS solution (2 μM COS in 40 mm sodium barbital-HCl, pH 8.4), resulting in an assay mixture containing 1.2 μM COS in a 25 mM buffer. We excluded any significant alteration by photochemical formation, especially as UV light was absent in the experiments and any small production rate would not be significant for the actual concentrations. Overpressure was released by a syringe. The COS solution was generated by bubbling the buffer with 0.01% COS (0.1 mmol COS/mol N_2) according to the method described by Protoschill-Krebs et al. (1995). For the experiments on CA inhibition, 500 μm EZ (6-ethoxy-2-benzylthiazole-2-sulfonamide or ethoxyzolamide) was added to the assay to a final concentration of 108 μM. All incubations for the COS uptake determinations were performed under the identical photon flux density of 10 μmol photons m^{-2} s^{-1}. The influence of different light intensities during incubation was not tested, since it is well known that the key enzyme for the consumption of COS, carbonic anhydrase, is principally light independent.

Determination of sulfur compounds

The concentrations of the reduced sulfur compounds COS and H_2S were measured in the assay solution by using the slightly modified “dynamic

headspace method" of Rasmussen et al. 1982a) and Ferek and Andreae (1983), which is described in detail elsewhere (Protoschill-Krebs et al. 1996). According to this method, the reduced sulfur compounds were cryogenically trapped and then analyzed by gas chromatography following the method described by Hofmann et al. (1992). The calibration including the determination of the losses in the dynamic headspace system was performed according to Protoschill-Krebs et al. (1996).

Chlorophyll determination

The chlorophyll (Chl) content of the algal assay samples was determined by the analysis of Chl a and b (*M. squamata*) and Chl a and c (*P. parvum* and *A. klebsii*), according to a slightly modified method of Shoaf and Lium (1976). An aliquot of the algal solution was filtered through a glass fiber filter (Schleicher & Schüll, No. 6, 2 cm diameter). The filter was then placed into an ampule (8.5 cm^3) and completely covered by 4 cm^3 of a DMSO-acetone solution (1:3). The Chl was extracted from the cells during an incubation period of 16 h at 4 C. The Chl in this solution was then photometrically measured at 20 °C at the wavelengths 664 nm and 630 nm (for the Chl a and c containing algae *A. klebsii* and *P. parvum*), and 663 nm and 645 nm (for the Chl a and b containing alga *M. squamata*) with 750 nm as a reference wavelength. The Chl content was calculated according to Shoaf and Lium (1976) for the Chl a and b containing algae and according to Jeffrey and Humphrey (1975) for the Chl a and c containing algae. Even though the equation in the latter work was obtained for chlorophyll dissolved in 9:1 acetone-water solution, it can be used here as the absorption spectrum of chlorophyll in the DMSO-acetone solution was shown to be quantitatively identical to that in 9:1 acetone-water.

Determination of the cell number

Cell numbers of the algal assay samples were determined with a cell counter (Coulter Counter, Coulter Electronics Inc., U.S.A.). The counter was calibrated with 5 μm latex beads. The algal suspension was diluted with a filtered (0.2 μm pore size) NaCl solution (28 kg/m^3). The medium without algae was treated likewise and its background value subtracted. The cell number was estimated as the sum of all particles larger than 1.6 μm.

Results and discussion

All algae were grown under low-light conditions (i.e., 10 μmol photons m^{-2} s^{-1}), and in addition, *M. squamata* was grown in thermostat cultures under

high-light conditions (100 μmol photons m^{-2} s^{-1}) were tested. Since the algae were growing well under the low-light intensity, we assume that the CO_2 transport mechanisms, which might also be involved in the transport of COS, were operating. Therefore, we conclude that the cell membranes did not represent a significant resistance for the COS uptake and consumption. Hence, the studies should permit general discussions about the COS consumption by the phytoplankton species investigated.

All three marine species tested (*A. klebsii, M. squamata,* and *P. parvum*) consumed COS from their surrounding medium (Table 1). As shown for *M. squamata* (Figure 1), the COS concentration in the reaction vial decreased significantly in the presence of the algae, whereas the COS decrease in the control without algae, due to chemical hydrolysis, was extremely slow. Furthermore, increasing the number of algal cells by a factor of 3 also enhanced the consumption of COS by the same factor. At higher algal concentrations, the decrease of the COS concentration slowed down after 3 min owing to the reduced COS concentration in the vial, caused by the COS uptake by the algae.

Table 1 shows that the COS uptake rates of the different algal species can vary significantly, depending on growth conditions and species (Table 1). Inhibition of carbonic anhydrase by a lack of the essential trace element zinc can be excluded since both media of the tested algal species contained enough zinc to ensure that carbonic anhydrase was not inhibited. High-light conditions led to a decrease in the consumption by *M. squamata* (Figure 2). The uptake of COS by this species was an order of magnitude higher when the algae were grown under low-light conditions (10 μmol photons m^{-2} s^{-1}) in batch cultures as compared to the uptake rate of algae grown under high-light conditions (100 μmol photons m^{-2} s^{-1}) in aerated thermostat cultures (see Materials and Methods). Pre-testing two batch cultures grown under different light intensities at 10 and 80 μmol photons m^{-2} s^{-1}, it was found that the photon flux density as such cannot be responsible for the observed differences of the COS consumption rates, which showed only small and nonsignificant differences of 20%. As the CA is a principally light independent enzyme, we assume pH differences and related CO_2 availability to be responsible for the different COS uptake rates. At the time the algae were harvested, the pH of the thermostat cultures was approximately 7.0 whereas the pH of the batch cultures was 8.4. Changing the pH, influences the quotient $[HCO_3^-/CO_2]$ in the medium as well. According to Enns (1967), the $[HCO_3^-/CO_2]$ ratio can be calculated by the simplified equation $pH = 6.1 + \log [HCO_3^-/CO_2]$. For a pH of 7.0 in the thermostat cultures this ratio is 8, whereas for the pH 8.4 of the batch cultures the ratio increases to 200. Hence, the availability of CO_2 for the batch culture algae is significantly lower, inducing an increasing

Table 1. Comparison of the COS uptake rate of various algal species. The numbers in parentheses indicate the number of experiments from which the mean value was calculated. B = batch culture; T = thermostat culture (see text). All COS exchange measurements were performed under low-light conditions (10 μmol photons m^{-2} s^{-1}).

Algal species	Growth conditions	COS uptake (n) [mg/(g Chl min)]	Reference
Amphidinium klebsii	10 μmol photons m^{-2} s^{-1} B	0.62 ± 0.2 (5)	This work
Prymnesium parvum	10 μmol photons m^{-2} s^{-1} B	0.41 ± 0.34 (4)	This work
Mantoniella squamata	100 μmol photons m^{-2} s^{-1} T	0.64 ± 0.23 (6)	This work
Mantoniella squamata	10 μmol photons m^{-2} s^{-1} B	7.35 ± 3.31 (12)	This work
Chlamydomonas reinhardtii	10 μmol photons m^{-2} s^{-1} T 3% CO_2 (30 mmol CO_2/mol $N_{2)}$	9.4	Protoschill-Krebs et al. 1995
Chlamydomonas reinhardtii	10 μmol photons m^{-2} s^{-1} T 0.03% CO_2 (0.3 mmol CO_2/mol N_2)	38	Protoschill-Krebs et al. 1995

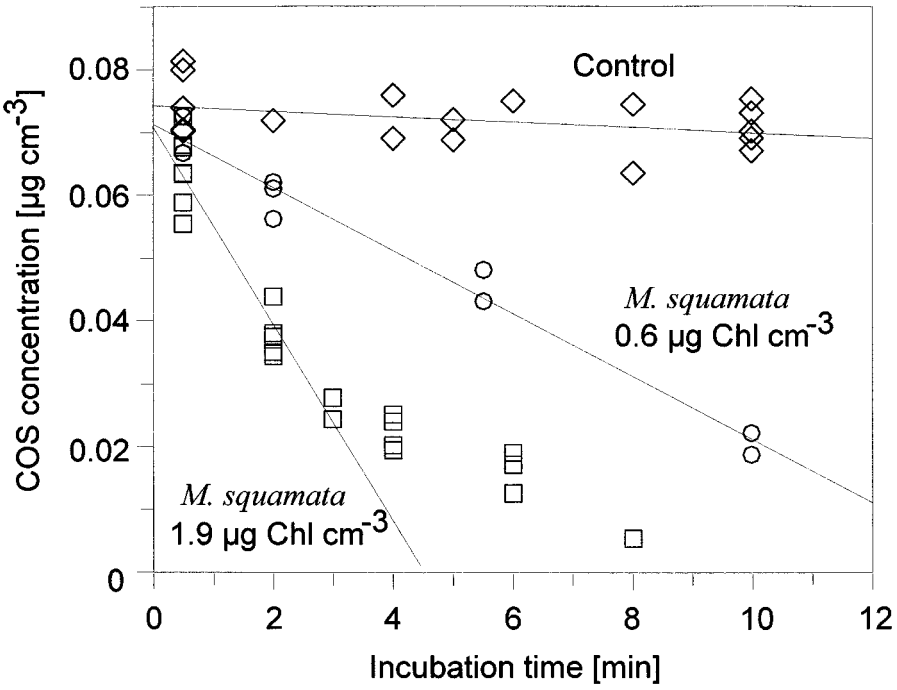

Figure 1. COS uptake by *M. squamata* under a photon flux density of 10 μmol photons m^{-2} s^{-1} shown as the decrease of the COS concentration with time in relation to the chlorophyll content of the assays. Open diamonds: control without algae; open circles: 0.6 μg Chl/cm^3; open squares: 1.9 μg Chl/cm^3. At higher algal concentrations, only the data obtained in the first three minutes of incubation were used for the calculation of the linear regression of the COS uptake.

need for a CO_2 concentrating system. This assumption is consistent with the results of Protoschill-Krebs et al. (1995) for *Chlamydomonas reinhardtii*, a freshwater alga, which shows significantly higher COS consumption rates when grown under a low CO_2 concentration as compared to algae grown under a high CO_2 concentration (Table 1 in Protoschill-Krebs et al. 1995). Therefore, we have to assume that under these different conditions the algae either synthesize or activate different amounts of CA. Since this enzyme catalyzes the hydrolysis of COS to yield CO_2 and H_2S, higher activities of CA under low-light conditions could explain the observed differences. We were not able to verify this assumption experimentally either by potentiometric (Wilbur & Anderson 1948) or by electrophoretic (Williams & Colman 1994) methods, since the CA content of *M. squamata* was found to be too low to allow the isolation and quantification of this enzyme (Conze 1995). However, as the enzyme CA was assumed to be responsible for the uptake of COS,

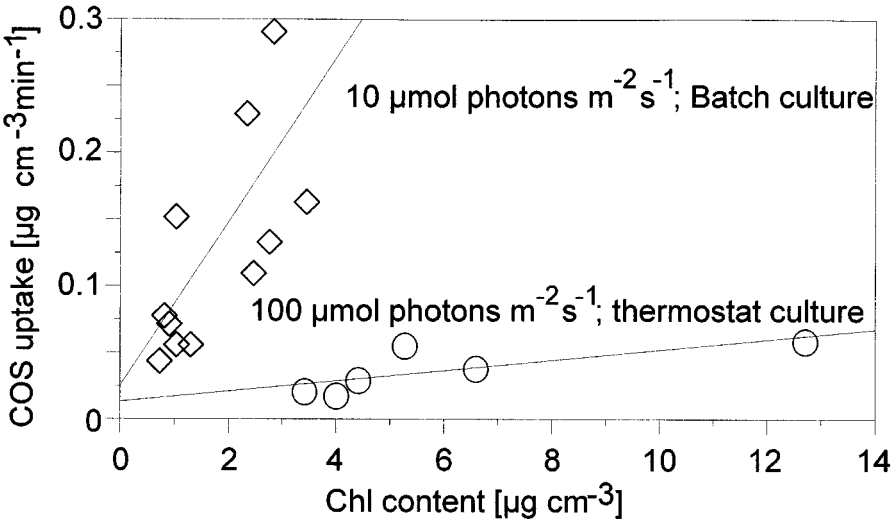

Figure 2. Comparison of the COS uptake by *M. squamata* grown under different culture conditions. Open diamonds: COS uptake by algae grown under 10 μmol photons m^{-2} s^{-1} in a batch culture. Open circles: COS uptake by algae grown under 100 μmol photons m^{-2} s^{-1} in an aerated thermostat culture.

we tested whether an inhibitor of CA could also inhibit the COS uptake by a marine alga. As shown in Figure 3, the addition of the CA specific inhibitor ethoxyzolamide (EZ) leads to a complete inhibition of the COS uptake by *M. squamata*. Therefore, it can be concluded that CA is the key enzyme for COS uptake and consumption by this species. This conclusion is of special interest as it shows that the COS uptake by algal cells might be a more sensitive test to detect CA activity.

Investigations of the concentration-dependent uptake of COS showed a clear increase of the COS uptake relative to an increase of the COS concentration in the incubation medium (Figure 4). As a consequence of the function of CA, we found a simultaneous production of H_2S, showing that COS is hydrolyzed to CO_2 and H_2S (Figure 4). Because H_2S is highly soluble in water, its quantification is more complicated than that of COS. Therefore, the estimation at low concentrations is difficult and the standard deviations of the measurements are high. Hence the slope of the linear regression is only a rough estimate and has been included to show that H_2S emission by the algae is of a magnitude similar to that of the COS uptake. From a double reciprocal transformation (Figure 5) of the COS graph (Figure 4), the $K_{1/2}$(COS) value, describing the affinity of the organism towards COS, was calculated to be 222 μM (222 mol/m^3). As compared to the value determined for the freshwater alga *C. reinhardtii*, $K_{1/2}$(COS) = 8.9 μM (Protoschill-Krebs et al. 1995), the

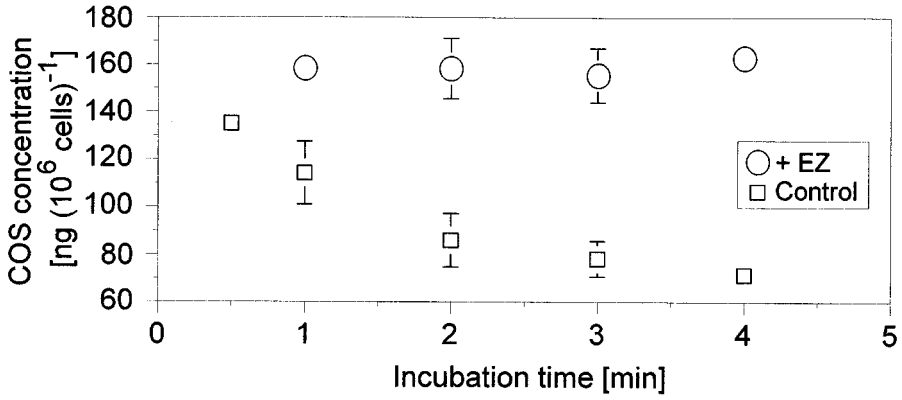

Figure 3. Effect of EZ, a CA inhibitor, on COS consumption by *M. squamata*. The COS uptake was determined in the presence of 108 μM EZ (open circles) and in the absence of EZ (open squares). Each point represents 2–4 measurements.

$K_{1/2}$(COS) of the marine alga is higher by a factor of 20. It is known that *C. reinhardtii* has a specific CO_2 concentration mechanism with extracellular CA (located in the periplasmatic space) which increases the availability of CO_2 for the alga. As a detection of any CA activity by a potentiometric assay (Conze 1995) was not possible, we assume that the marine algal species investigated do not possess extracellular CA, which could enhance COS uptake.

Within this context, it is of interest whether marine algae are significantly involved in the COS production and consumption in oceans. Although our experiments were performed under low-light intensities, we are able to roughly estimate the global algal contribution to the total COS flux. Basing our estimation on the linear dependence of the COS uptake on the COS concentration (as illustrated for *M. squamata* in Figure 4) and assuming a global average concentration of ca. 0.2 mg Chl m^{-3} (estimated by extrapolating data of Yoder et al. 1993), a mixing depth of 50 m (Lewis et al. 1988) and ca. 25 pM COS (estimated from data of Andreae 1990; Andreae & Jaeschke 1992), the global algal uptake of COS for an ocean surface of 361 $\times$ 10^6 km^2 was found to be around 12 $\times$ 10^{-3} pmol l^{-1} h^{-1}. In comparison to the photoproduction and hydrolysis rates of COS, 5 pmol l^{-1} h^{-1} and 1 pmol l^{-1} h^{-1}, respectively, estimated by Andreae and Ferek (1992) for the North Atlantic in April/May, the contribution of the algae to the global COS exchange is insignificant (<1%). Further studies are needed to more clearly elucidate the effects of different light intensities as well as the free zinc concentrations on CA for a conclusive final estimation. However, even with such an improvement, we expect that the role of marine phytoplankton

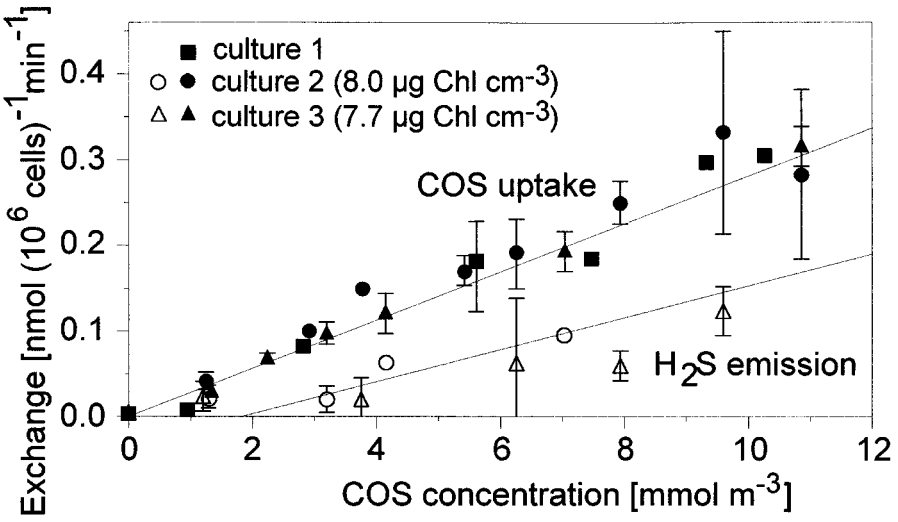

Figure 4. Dependency of the COS uptake (closed symbols) and the H_2S emission (open symbols) by *M. squamata* on the COS concentration in the surrounding medium. Data are given as mean values ± standard error from 2–4 measurements. For some data, the standard error is smaller than the symbol.

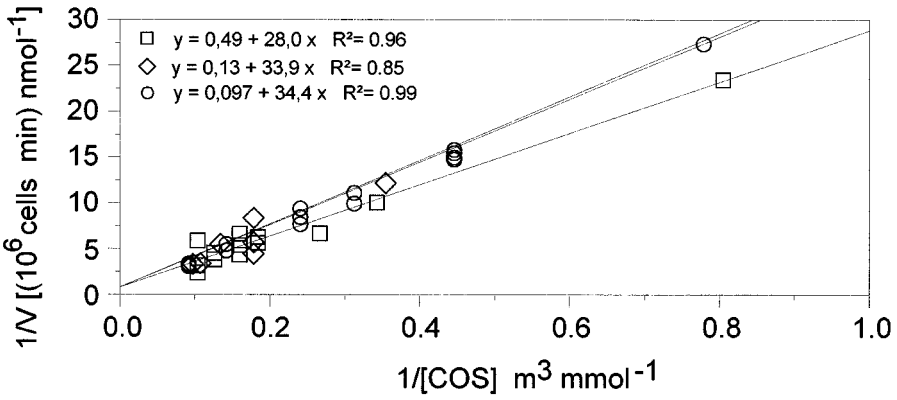

Figure 5. Lineweaver-Burk plot of the COS uptake by *M. squamata* for the determination of $K_{1/2}$ which represents the affinity of an organism towards a substrate. The reciprocal values of the consumption velocities (see Figure 3) are plotted against the reciprocal values of the substrate concentration. According to this procedure $-1/K_{1/2}$ is found at the intersect of the regression line with the x-axis.

will be confirmed to be a minor component within the global COS exchange between oceans and the atmosphere.

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