64

N91-31778

EFFICIENCY OF N USE BY WHEAT AS A FUNCTION OF INFLUX AND EFFLUX OF NO_3^-

R.C. Huffaker, M. Aslam, M.R. Ward, University of California, Davis, CA

ABSTRACT

Since N assimilation is one of the most costly functions of a plant, its efflux before assimilation results in a serious energy cost and loss in efficiency which could decrease yields. Efficient crop production is critical to CELSS. Our objective is to determine the extent of efflux of the N species NO_3^-, NH_4^+, NO_2^-, and urea after uptake, and possible means of regulation. We found that NO_3^- efflux became serious as its substrate level increased. Efflux/Influx (E/I) of NO_3^- was greater in darkness (35%) than in light (14%) and the ratio greatly increased with increased substrate NO_3^-, (up to 45% at 10 mM). It seems advantageous to use the lowest possible nutrient concentration of NO_3^-. The feasibility of using ClO_3^- as a trapping agent (competitive inhibitor of NO_3^- uptake) for effluxed NO_3^- was assessed and its toxicity determined.

INTRODUCTION

Crop production during extended space flights requires the development of procedures leading to the optimum use of the available energy. Optimizing the utilization of N by crop plants represents an area where significant progress can be made in the CELSS program.

It is well recognized that ion uptake requires ATP and electron flow. The cost of NO_3^- assimilation is particularly high not only due to the energy requirement of uptake but also because of the need for 10 electrons to reduce it to the level of glutamate. In addition are the costs of maintaining pH balance, since each reduction of NO_3^- to the level of NH_4^+ produces an OH^-. Thus it is not surprising that estimates of respiratory costs of ion absorption range up to 50% of total root respiration and 20% of total plant respiration (1).

Recent reports in the literature indicate that NO_3^- efflux can begin very rapidly after its influx (2,3,4,5,6). In addition, prolonged leakage of NO_3^- occurs from root storage pools dependent upon the N-status of the roots

(7,8,9,10,11). Lee and Clarkson (6) estimated that efflux, at external concentrations above 1 mM, could account for up to 40% of the influxed NO_3^-. Thus NO_3^- efflux could represent a significant additional cost to an already energy costly assimilatory pathway. Little is known concerning the regulation of NO_3^- efflux. Recent work of Briskin (12) showed evidence that efflux of NO_3^- utilizes ATP, which could greatly add to the energy cost of NO_3^- assimilation.

Measurement of NO_3^- efflux has been hampered by the lack of easy, rapid, and less costly analytical techniques for detecting the very low concentrations involved in a process with a half-life ($t_{1/2}$) of minutes. The use of ^{15}N as a tracer is laborious and has problems of sensitivity. ^{13}N has only a 10 min half-life, must be used at the site of generation (cyclotron), and is very costly to use. We developed an HPLC method which has the sensitivity, ease and low cost required. In addition, we evaluated ClO_3^- as a trapping agent for NO_3^-.

This report presents results estimating NO_3^- influx, efflux, and net uptake across several mechanisms of NO_3^- uptake.

MATERIALS AND METHODS

Plant growth. Wheat seedlings were grown hydroponically in a 1/4 strength Hoagland's solution for 8 days in an environmentally controlled growth chamber at 400 $\mu E/m^2 sec$, at 18°C, and 80% relative humidity (13,14). On the 7th day they were transferred into 1/4 strength Hoagland's (loading solution) containing NO_3^- at the concentration to be used in the efflux study (specified in each experiment).

Measurement of NO_3^- efflux. After removal from the loading solution, 10 seedlings were placed in 300 ml of the efflux solution for various periods of time (specified below) containing 0.06 mM Pi at pH 5.8, 0.2 mM $CaSO_4$, and with or without ClO_3^- at the specified concentrations. The seedlings were rinsed

for 2 sec in 300 ml of efflux solution, then placed in 60 ml of efflux solutions for the following times: 10 sec, 30 sec, 1, 2, 5, 10, 15, and 20 min and the amount of NO_3^- released at each time from the roots was determined.

Measurement of NO_3^- and ClO_3^-. These compounds were measured by HPLC as described previously for NO_3^- (13,14). ClO_3^- was also measured at 210 nm with a UV monitor.

Uptake rates of NO_3^- and ClO_3^-. Uptake rates were determined as previously described by determining rates of depletion of NO_3^- from substrate solutions, then fitting the rate curves to best fit curves by polynomial analysis using a computer (13).

RESULTS

Mechanisms of NO_3^- uptake. The results in Figures 1 and 2 show several mechanisms for NO_3^- uptake. Uptake as a function of NO_3^- concentration can be determined by either step up or step down, on continuous depletion experiments; Figures 1 and 2 are the results of continuous depletion experiments. In Figure 1, one mechanism is readily seen between about 0.2 and 0.7 mM. This is commonly referred to as Mechanism I in the literature (14). The rates above 0.7 mM are largely undefined but are referred to in the literature as Mechanism II. Another mechanism is indicated in Figure 1 at concentrations below 0.1 mM and it is readily seen when the data are plotted between 0 and 0.1 mM (Fig. 2).

Comparison of uptake of NO_3^- and ClO_3^-. The comparative uptake of NO_3^- and ClO_3^- is shown in Figures 3 and 4 at initial concentrations of 0.5 and 1 mM. Wheat plants deplete the NO_3^- concentration very efficiently to near zero (Fig. 1), whereas depletion of ClO_3^- is not straight forward. The ability to take up ClO_3^- is continuously lost with time (Fig. 4).

Toxic effects of ClO_3^- on NO_3^- uptake. The increasingly toxic effects of ClO_3^- on NO_3^- uptake with time is seen in Table 1.

Comparative effects of pretreatments of ClO_3^- and NO_3^- on their uptakes. The comparative effects of pretreatments of ClO_3^- and NO_3^- on their uptakes are shown in Table 2. Pretreatments varying in time had little effect on subsequent uptake of NO_3^-, whereas increasing time of pretreatments greatly decreased ClO_3^- uptake.

ClO_3^- as a competitive inhibitor of NO_3^- uptake. Double reciprocal plots of rates vs concentrations show evidence that ClO_3^- is a competitive inhibitor of NO_3^- uptake (Fig. 5).

NO_3^- efflux. Figure 6 shows a typical example of a determination of NO_3^- efflux. Our results matched quite closely those reported in the literature showing two different early losses of NO_3^-, one with a $t_{1/2}$ of less than 10 sec and another with a $t_{1/2}$ of minutes. After one min, the second set of rates approximate an apparent first order reaction. Extrapolation to t_0 gives an estimate of the rate of NO_3^- efflux. At a concentration of 1 mM NO_3^-, efflux varied consistently between 2.0 and 2.5 μmol/gxh.

Effect of increasing concentrations of NO_3^- and ClO_3^- on NO_3^- efflux. Efflux in the presence of increasing concentrations of NO_3^- and ClO_3^- is shown in Figure 7. As expected, efflux increased with increasing concentrations of NO_3^- and ClO_3^-.

Influx, efflux, and net uptake of NO_3^-. Comparative rates of the three kinetic components of NO_3^- absorption are shown in Table 3. Efflux and influx greatly increased between 0.2 and 10 mM external NO_3^-, whereas net uptake remained about the same. Efflux/influx increased from 15 to 45% with increasing concentration of NO_3^-.

Effect of light and dark on influx, efflux, and net uptake of NO_3^-. NO_3^- efflux was similar in plants in darkness and in light; however, influx and net uptake were much greater in light (Table 4). Thus, in darkness 35% of the influxed NO_3^- was effluxed, while in light the proportion effluxed was reduced to 14%.

DISCUSSION

It is important to continue to develop information concerning the mechanisms of uptake of nutrient ions both to understand the reactions of the plants to changing concentrations and also for planning optimum concentrations of nutrient solutions for maximum efficiency.

Mechanisms of NO_3^- uptake. At least three mechanisms of NO_3^- uptake are present, one between 0 and about 0.05 to 0.08 mM with a K_m of ca 0.012 to 0.018 mM, and another between 0.1 and about 0.7 mM with a k_m of ca 0.025 to 0.04 mM (Figs. 1 and 2). The latter is the typical mechanism reported in the literature (14). At concentrations above 1 mM, the mechanisms are largely undefined and are difficult to determine because efflux becomes such an important component (discussed below).

Comparison of uptake of NO_3^- and ClO_3^-. In a determination of NO_3^- efflux, very low concentrations of NO_3^- are present in the external solution. At these low levels of NO_3^- (see Fig. 3), the wheat plant can very efficiently absorb the NO_3^- as it is effluxed into the external solution. Hence, ClO_3^- has been used as a trapping agent for the effluxed NO_3^-.

Although much work has been reported on the effects of ClO_3^- on NO_3^- uptake, the analytical procedures reported to separate ClO_3^- from NO_3^- in the solutions were in some cases non specific, i.e., ion electrodes (3). Radioactive ClO_3^- was also used (3) which presents problems of low specific activity and sticking to glassware. We developed an HPLC method which effectively separates NO_3^- from ClO_3^- and both can be measured simultaneously. In addition, much of the reported literature did not discriminate between kinetic effects and toxic effects of ClO_3^- on NO_3^- uptake (15).

Toxic effects of ClO_3^-. The results showed that toxicity symptoms, as shown by decreased rates of uptake of NO_3^- and ClO_3^-, were apparent after 1 h (Fig. 4, Tables 1 and 2). Toxic effects of ClO_3^- were greater toward its own

uptake than towards NO_3^- uptake. We found that little toxicity occurred during the 20 min period of the efflux experiments.

ClO_3^- as a competitive inhibitor of NO_3^- uptake. The results verified that ClO_3^- was a competitive inhibitor of NO_3^- uptake as earlier reported (3) (Fig. 5). Apparently the NO_3^- transporter discriminated effectively between NO_3^- and ClO_3^- since proportionately much larger concentrations of ClO_3^- were required to inhibit NO_3^- uptake. In summary, ClO_3^- could be used as a trapping agent for effluxed NO_3^- for short time experiments.

NO_3^- efflux. The method for determining efflux was based on some excellent work done by Lee and Clarkson (6) and Shone and Flood (16) who used cereal roots to determine the kinetic parameters required to measure efflux of NO_3^-. They found that the combined $t_{1/2}$ of release of NO_3^- in the surface film attached to the roots from the external solution and from the root free space was ca 7 sec. In addition, their results showed that the $t_{1/2}$ for cytoplasmic release of NO_3^- was ca 4 min. A semilog plot of efflux rate vs time resulted in a linear regression line after 1 min, since at that time the first two kinetic parameters had passed through ca 9 half-lives. In our results, the relative contributions of these parameters would be very small in relation to the amount of NO_3^- efflux. The rates of efflux we measured are similar to those reported for cereals by workers using ^{13}N-NO_3^- at NO_3^- concentrations of 1.5 to 5 mM (3,4,6).

Effect of increasing concentrations NO_3^- and ClO_3^- on NO_3^- efflux. As expected, efflux increased with increasing concentrations of NO_3^- (Fig. 7). In the region of Mechanism II of uptake (0.2 mM), efflux was a significant deterrent to N use efficiency. Here 15% of the NO_3^- influxed was effluxed. Net NO_3^- uptake remained quite constant as external NO_3^- increased beyond 0.2 mM. This occurred because, although influx increased, efflux correspondingly increased. The ratio of efflux/influx, expressed as a percentage, increased up to 45% at 10 mM NO_3^-. These results help explain results from Raper's

laboratory which showed that net uptake changed little as NO_3^- concentrations increased to high levels (11). These workers also presented evidence for significant levels of efflux at increasing concentrations of NO_3^-.

Effect of light and dark on influx, efflux, and net uptake of NO_3^-. Since efflux is a function of the concentration of NO_3^- in the cytoplasm, light and dark periods had a profound effect on the ratio of efflux/influx (Table 4). Although efflux was the same in light or dark, influx was greatly increased in light along with the rate of NO_3^- reduction (17,18). Therefore, the relative percentage of NO_3^- effluxed is much less in light.

Effect of increasing concentrations ClO_3^- on NO_3^- efflux. Higher rates of NO_3^- efflux were detected as ClO_3^- increased in the efflux solution (Fig. 7). This could be because of ClO_3^- serving as a trapping agent or because of an unknown effect. The increase in efflux rate with 5 and 10 mM ClO_3^- was sufficiently large to be somewhat uncertain. We are currently evaluating this result.

Effect of NH_4^+ on NO_3^- efflux. The literature is confusing on this issue with results of little if any effect of NH_4^+ on NO_3^- efflux (5,6), and of large effect (4). We have not yet examined the interactions of the different N species on NO_3^- efflux.

Ramifications. As the concentration of NO_3^- increases in the external solution, efflux becomes an increasingly important energy cost to the plant. Not only is energy needed for NO_3^- influx, it now appears that NO_3^- efflux may utilize ATP (12). This results in an almost doubling of the cost of absorption at higher concentrations of NO_3^-. On this basis, it would seem that a nutrient solution for crop growth in CELSS should be optimized at the lowest concentrations possible. In addition, it is increasingly critical to determine the regulation of efflux of N compounds since it is not known how NO_3^-, NH_4^+, NO_2^-, and urea influence each others efflux. Will total efflux

decrease if the total concentration of N in the nutrient solution is made up of the four species instead of only NO_3^- and NH_4^+?

REFERENCES

1. Veen BW 1980 Energy cost of ion transport. *In:* Genetic engineering of osmoregulation. Eds, DW Rains, RC Valentine, A Hollaender. Plenum Press, New York, NY

2. Deane-Drummond CE, ADM Glass 1982 Nitrate uptake into barley (*Hordeum vulgare*) plants. A new approach using $^{36}ClO_3^-$ as an analogue for NO_3^-. Plant Physiol 70:50-54

3. Deane-Drummond CE, ADM Glass 1983a Short-term studies of nitrate uptake into barley plants using ion-specific electrodes and $^{36}ClO_3^-$. I. Control of net uptake by NO_3^- efflux. Plant Physiol 73:100-104

4. Deane-Drummond CE, ADM Glass 1983b Short-term studies of nitrate uptake into barley plants using ion-specific electrodes and $^{36}ClO_3^-$. Regulation of NO_3^- efflux by NH_4^+. Plant Physiol 73:105-110

5. Glass ADM, RG Thompson, L Bordeleau 1985 Regulation of nitrate influx in barley. Studies using $^{13}NO_3^-$. Plant Physiol 77:379-381

6. Lee RB, DT Clarkson 1986 Nitrogen-13 studies of nitrate fluxes in barley roots. I. Compartmental analysis from measurements of ^{13}N efflux. J Exptl Bot 37:1753-1767

7. Jackson WA, KD Kwik, RJ Volk 1976a Nitrate uptake during recovery from nitrogen deficiency. Physiol Plant 36:174-181

8. Jackson WA, KD Kwik, RJ Volk, RG Butz 1976b Nitrate influx and efflux by intact wheat seedlings: effects of prior nitrate nutrition. Planta 132:149-156

9. Lee RB, MC Drew 1986 Nitrogen-13 studies of nitrate fluxes in barley roots. II. Effect of plant N-status on the kinetic parameters of nitrate influx. J Exptl Bot 37:1768-1779

10. MacKown CT, WA Jackson, RJ Volk 1982 Restricted nitrate influx and reduction in corn seedlings exposed to ammonium. Plant Physiol 69:353-359

11. Tolley-Henry L, CD Raper, TC Granato 1988 Cyclic variations in nitrogen uptake rate of soybean plants: effects of external nitrate concentration. J Exptl Bot 39:613-622

12. Briskin DP 1988 Nitrate transport in plasma membrane vesicles from red beet (*Beta vulgaris* L.) storage tissue. Plant Physiol (Supple) 86:475

13. Goyal SS, RC Huffaker 1986a A novel approach and a fully automated microcomputer-based system to study kinetics of NO_3^-, NO_2^-, and NH_4^+ transport simultaneously by intact wheat seedlings. Plant, Cell and Environ 9:209-215

14. Goyal SS, RC Huffaker 1986b The uptake of NO_3^-, NO_2^-, and NH_4^+ by intact wheat (*Triticum aestivum*) seedlings. I. Induction and kinetics of transport systems. Plant Physiol 82:1051-1056

15. Guy M, G Zabala, P Filner 1988 The kinetics of chlorate uptake by XD tobacco cells. Plant Physiol 86:817-821

16. Shone MGT, AV Flood 1985 Measurement of free space and sorption of large molecules by cereal roots. Plant, Cell and Environ 8:309-315

17. Aslam M, RC Huffaker 1982 *In vivo* nitrate reduction in roots and shoots of barley (*Hordeum vulgare* L.) seedlings in light and darkness. Plant Physiol 70:1009-1013

18. Aslam M, RC Huffaker 1984 Dependency of nitrate reduction on soluble carbohydrates in primary leaves of barley under aerobic conditions. Plant Physiol 75:623-628

Table 1. Effect of pretreatment of ClO_3^- on NO_3^- uptake. The initial concentration of substrate NO_3^- was 0.6 mM.

Hours Pre-treatment	$[ClO_3^-]$ 1 mM	5 mM
	NO_3^- Uptake (μmol/gxh)	
0	7.3	7.3
1	6.2	5.3
2	5.3	3.0
3	4.9	2.3

Table 2. Effect of pretreatment of ClO_3^- or NO_3^- on their uptakes.

Hours Pre-treatment	Pretreatment (1 mM)	
	NO_3^-	ClO_3^-
	Uptake (at 1 mM) (μmol/gxh)	
0	6.3	3.5
3		1.1
5	6.8	0.5

Table 3. Efflux, net uptake and influx of NO_3^-. See Materials and Methods for procedures.

EFFLUX, INFLUX, AND NET UPTAKE OF NO_3^-

$[NO_3^-]$ (mM)	Efflux	Net Uptake	Influx	Efflux Influx
		μmol/gxh		%
0.2	1.1	6.5	7.6	15
1.0	2.5	8.5	11.0	23
10.0	7.7	9.5	17.2	45

Table 4. Effect of light and dark treatments (24 h) on NO_3^- efflux, net uptake and influx. See Materials and Methods for procedures.

Treatment	Efflux	Net Uptake	Influx	Efflux
		(μmol/gxh)		%
Light*	1.3	7.9	9.2	14
Dark	1.0	1.8	2.8	35

*plants induced in 0.2 mM NO_3^- for 24 h light or dark.

FIGURE LEGENDS

Fig. 1. NO_3^- uptake from 0 to 1000 μm. See Materials and Methods for procedures.

Fig. 2. NO_3^- uptake from 0 to 100 μm. See Materials and Methods for procedures.

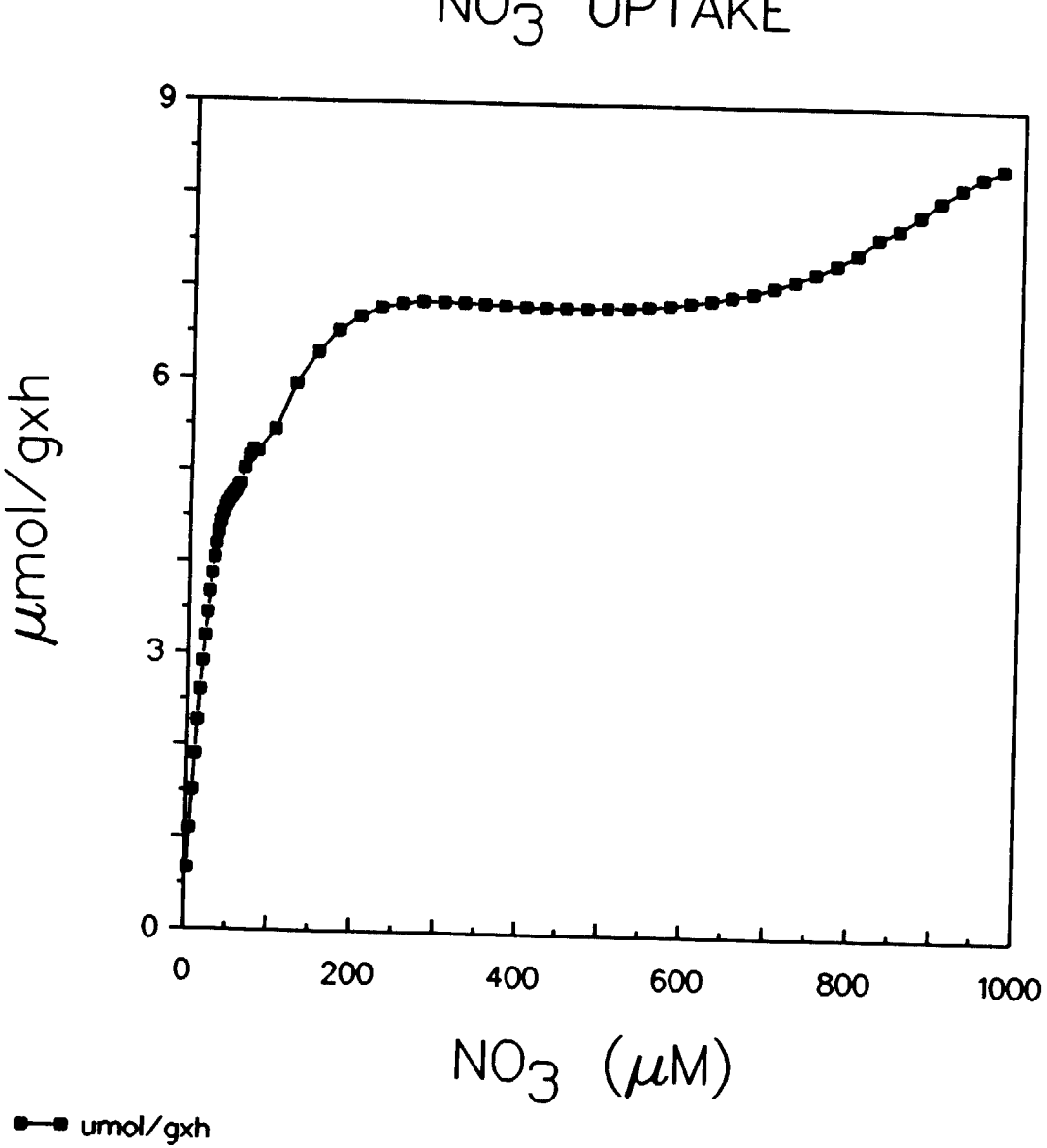
Fig. 3. Depletion of NO_3^- from 0.5 and 1.0 mM substrate solutions. See Materials and Methods for procedures.

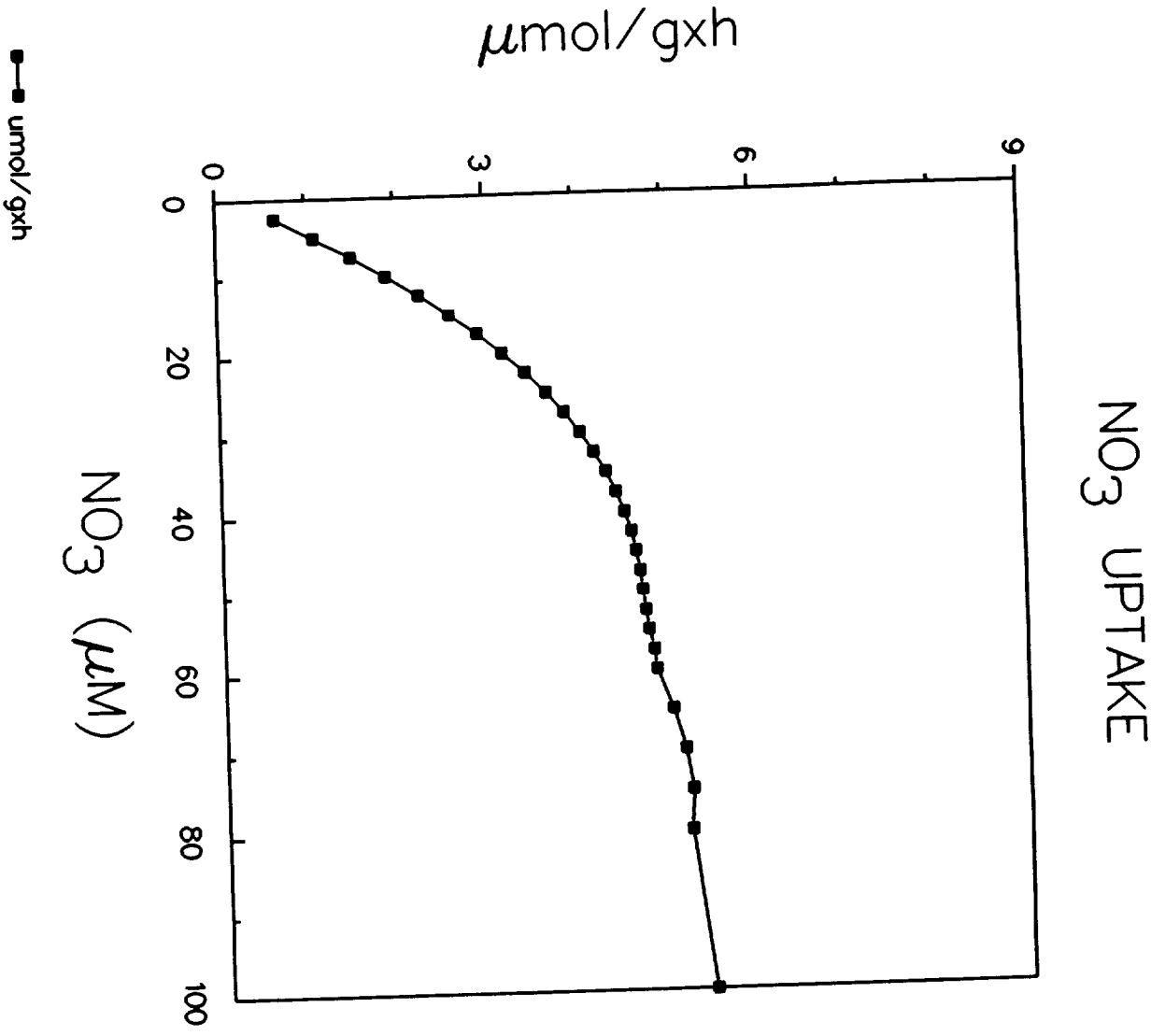
Fig. 4. Depletion of ClO_3^- from 0.5 and 1.0 mM substrate solutions. See Materials and Methods for procedures.

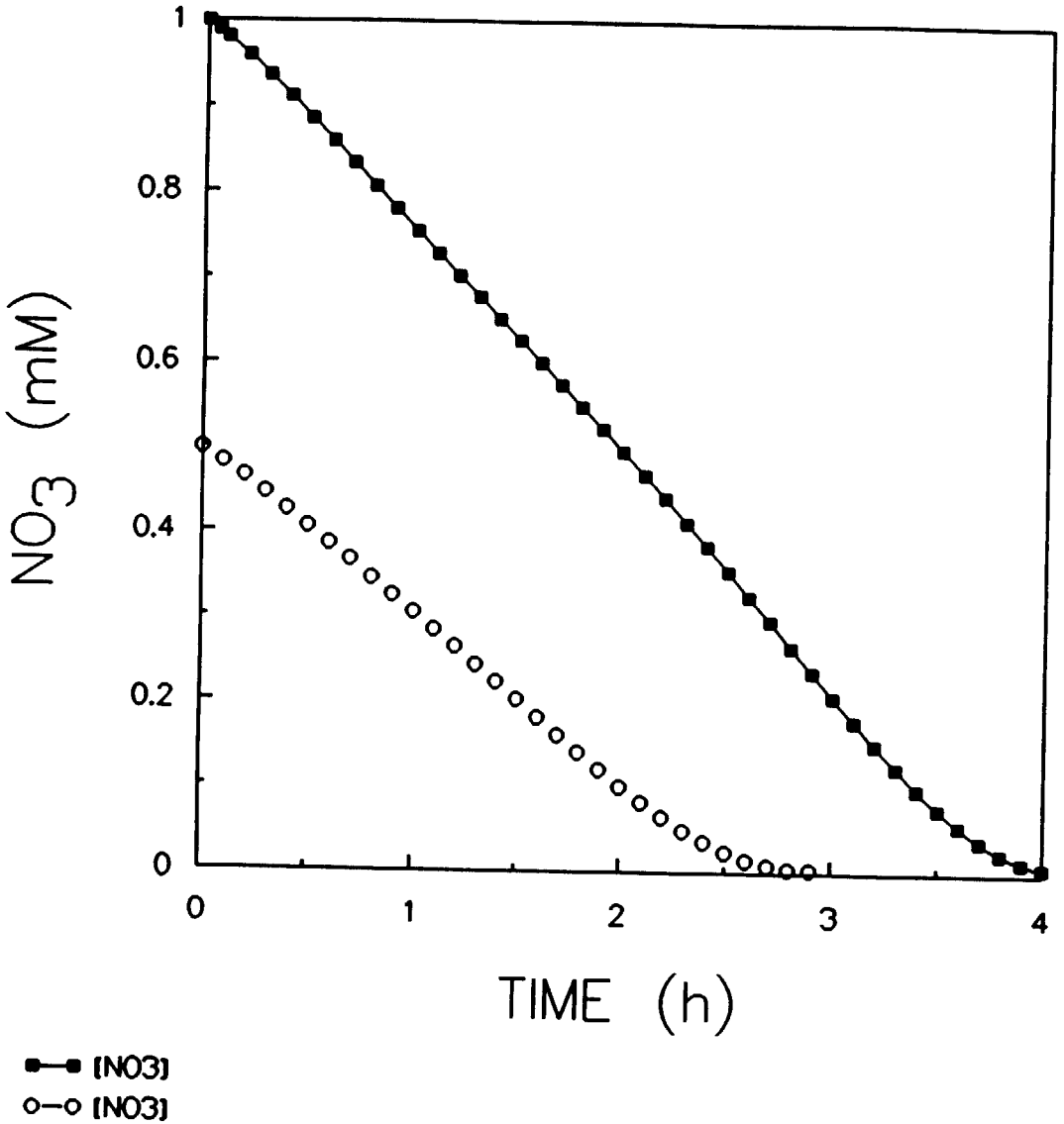
Fig. 5. ClO_3^- as a competitive inhibitor of NO_3^- uptake. See Materials and Methods for procedures.

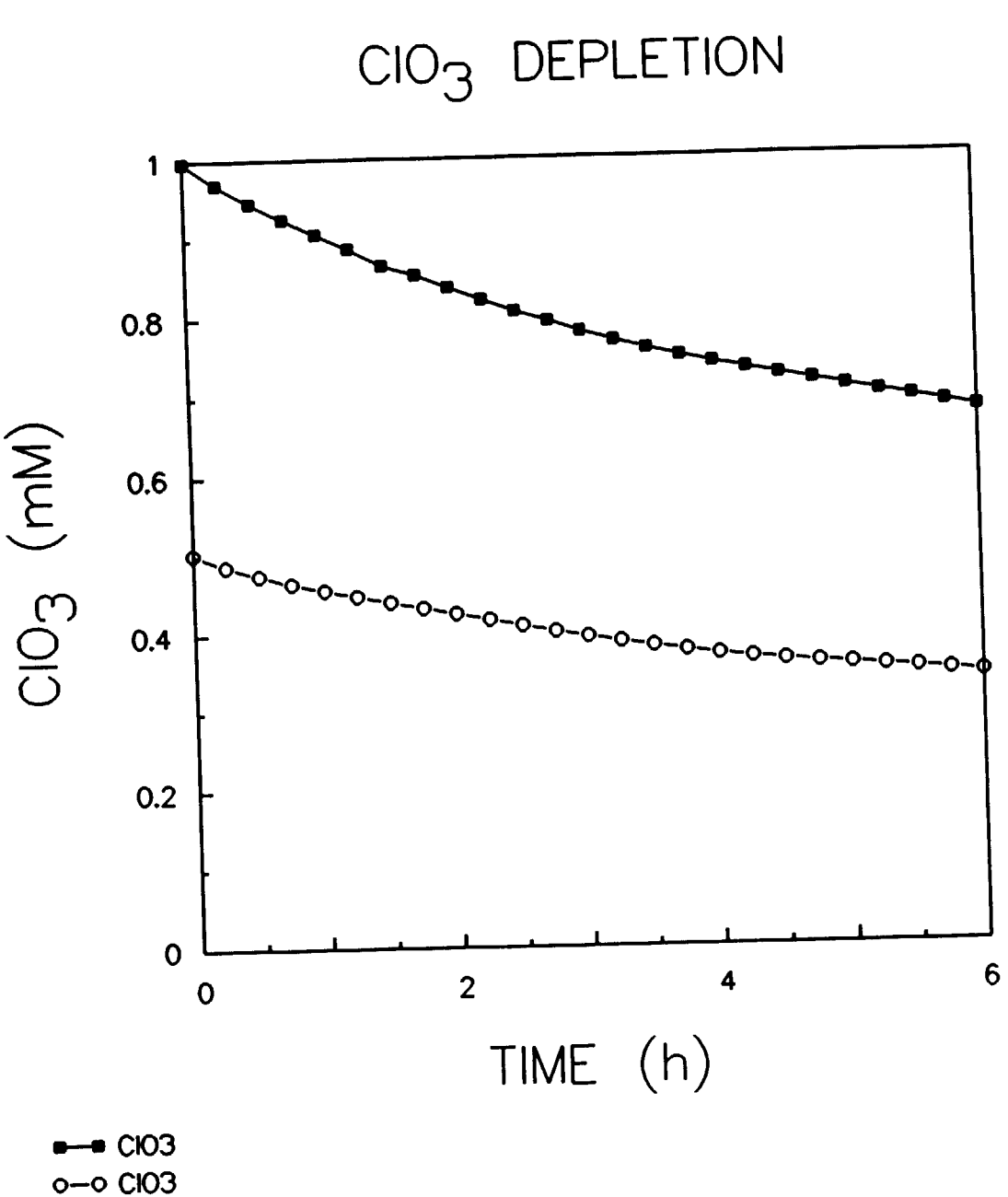
Fig. 6. Semilog plot of NO_3^- efflux vs time. See Materials and Methods for procedures.

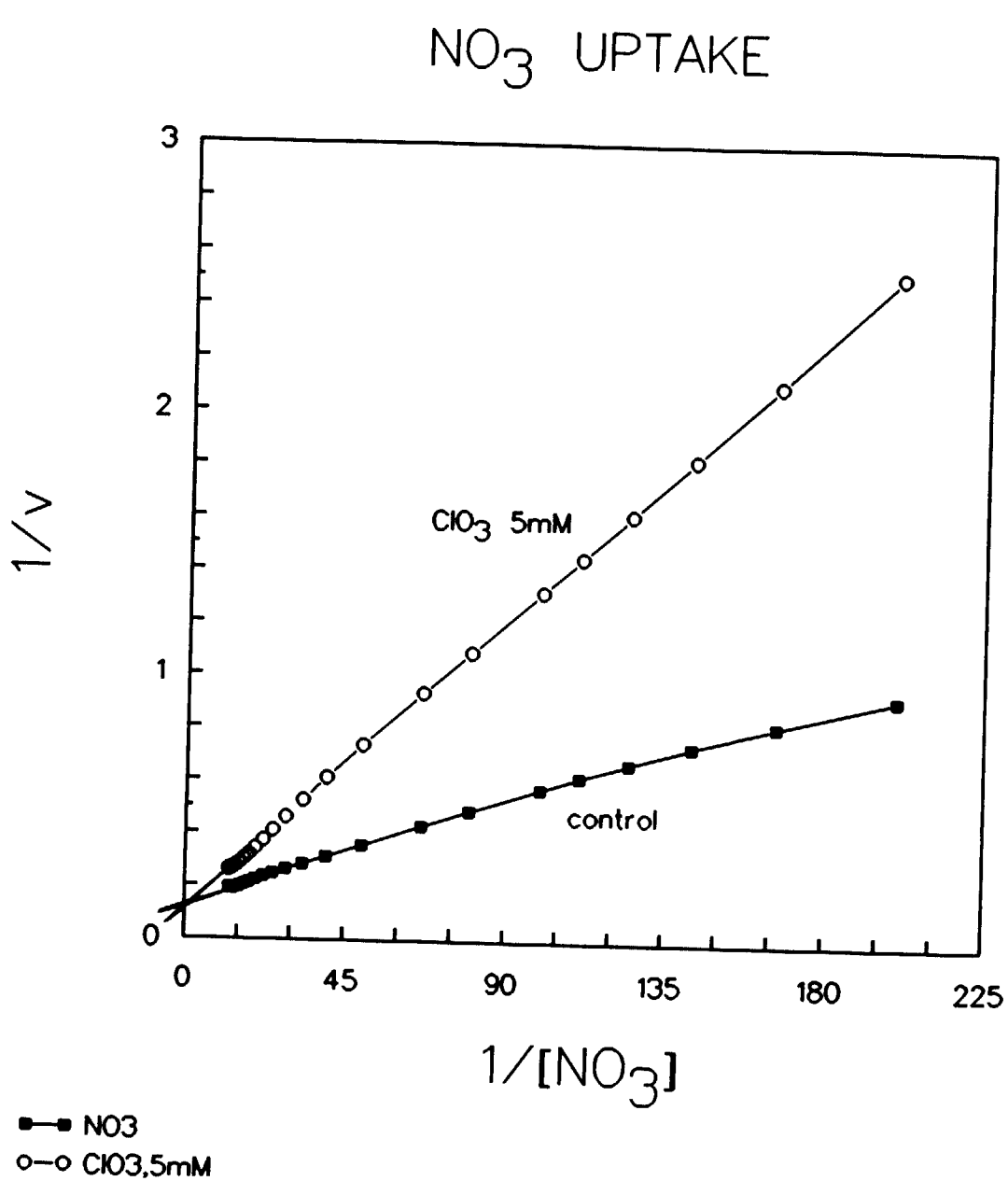
Fig. 7. Effect of ClO_3^- on NO_3^- efflux. See Materials and Methods for procedures.

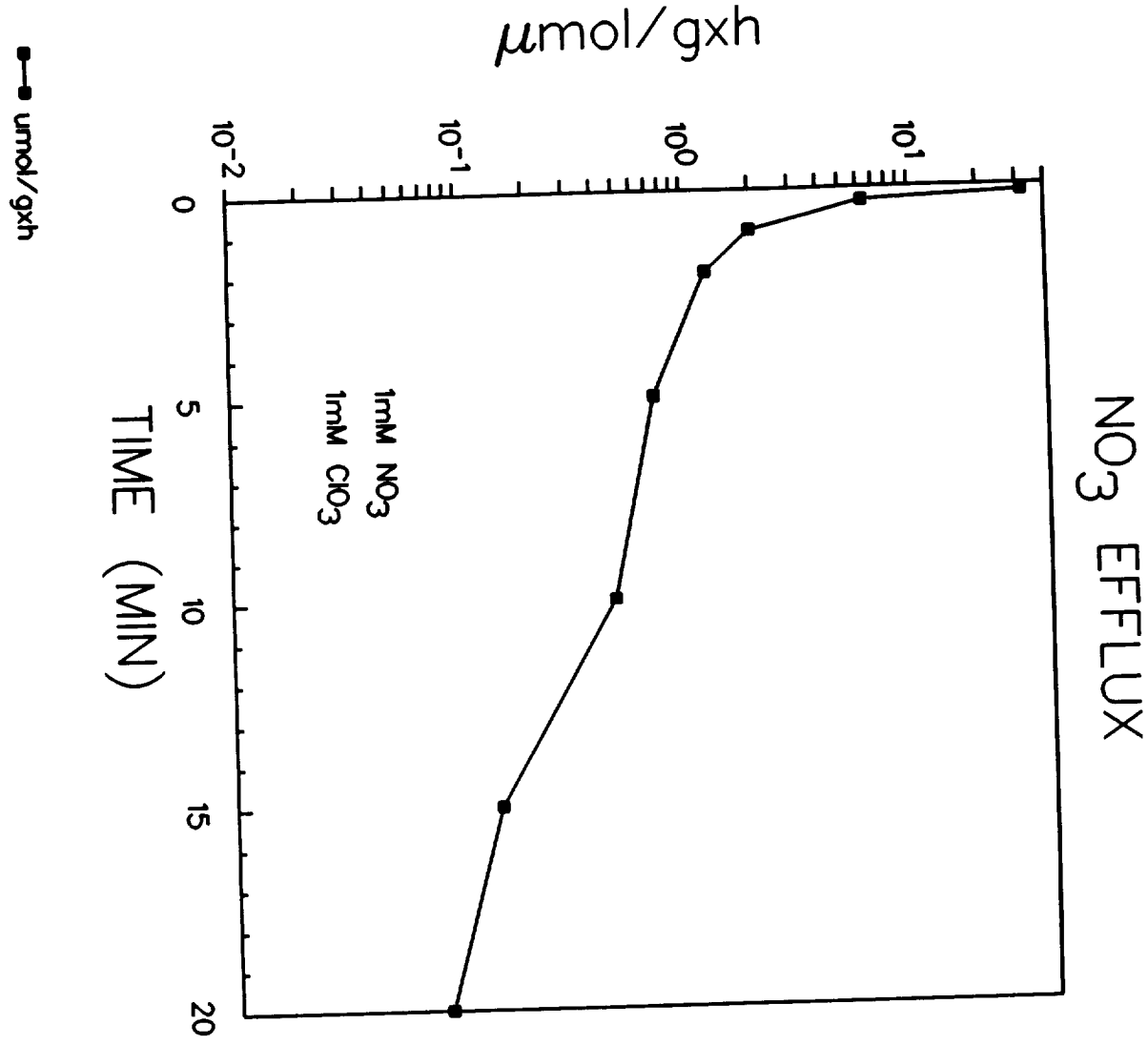

NO3 UPTAKE
9
6
3
0
μmol/gxh
0
200
400
600
800
1000
NO3 (μM)
umol/gxh

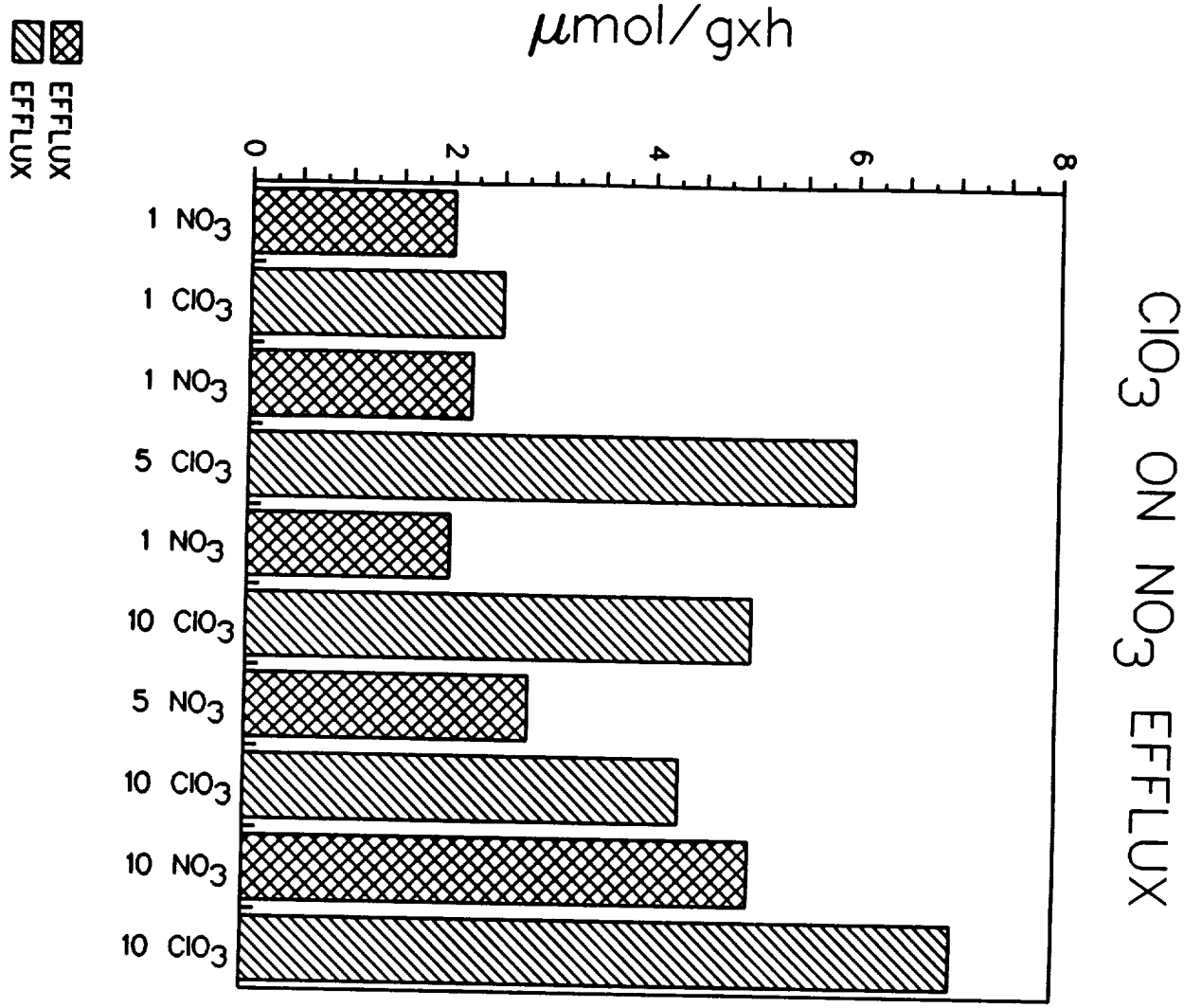

μmol/gxh
0
3
6
9
NO3 UPTAKE
0
20
40
60
80
100
NO3 (μM)
umol/gxh


NO3 DEPLETION
NO3 (mM)
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
TIME (h)
[NO3]
[NO3]


ClO3 DEPLETION
ClO3 (mM)
1
0.8
0.6
0.4
0.2
0
0
2
4
6
TIME (h)
ClO3
ClO3


NO3 UPTAKE
3
2
1
0
1/v
ClO3 5mM
control
0
45
90
135
180
225
1/[NO3]
NO3
ClO3,5mM


μmol/gxh
10^{-2}
10^{-1}
10^{0}
10^{1}
0
5
10
15
20
TIME (MIN)
1mM NO_3
1mM ClO_3
NO_3 EFFLUX
umol/gxh


EFFLUX
EFFLUX
μmol/gxh
0
2
4
6
8
1 NO3
1 ClO3
1 NO3
5 ClO3
1 NO3
10 ClO3
5 NO3
10 ClO3
10 NO3
10 ClO3
ClO3 ON NO3 EFFLUX