Fate of Cyanide and Related Compounds in Industrial Waste Treatment

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INTRODUCTION

Cyanide removal by biological oxidation has been the subject of considerable research over the past forty years. Apparent removal has been demonstrated in both trickling filters (7) and activated sludge units (12) although cyanide is toxic to biological systems (5). Reports indicate that cyanide concentrations of about 2 mg/l (as HCN) are toxic to unacclimated bacterial cultures, but much higher cyanide concentrations can be tolerated and actually removed from solution by acclimated cultures. Most researchers report that ammonia production accompanies the apparent cyanide removal. In most instances, a supplemental carbon source such as glucose, raw sewage, or soluble fish food was fed to the biological oxidation units along with cyanide.

Recently, biochemists working with pure cultures of Bacillus megaterium have demonstrated a pathway for bacterial metabolism of cyanide via an intermediate, β-cyanoalanine (4). Initial work indicates that cyanide probably reacts with an amino acid such as serine to form β-cyanoalanine and is eventually incorporated into asparagine or other protein precursor. It is not clear whether

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cyanide enters the cell as HCN or as a reaction product of some extracellular reaction.

Researchers investigating the biological oxidation of cyanide face a difficult task since cyanide is highly volatile at neutral pH and highly reactive at alkaline pH. Failure to account for stripping of cyanide from solution and for possible reactions of cyanide with substrate could cause erroneous results. In certain instances, researchers have added caustic to feed solutions containing both carbohydrates and cyanide in order to prevent volatilization of the cyanide.

The objective of this paper is to determine the fate of cyanide in aerated biological waste treatment. Four interrelated possibilities exist for cyanide removal in an aerated biological system: 1) chemical reaction in solution with the substrate; 2) adsorption onto biological floc; 3) stripping, and; 4) biological metabolism. Each of these possibilities was investigated to determine its relative importance with regard to overall cyanide removal.

CHEMICAL REACTION WITH SUBSTRATE

Hydrogen cyanide forms in water whenever soluble NaCN or KCN salts are added, and dissociates as follows:

$$HCN \rightleftharpoons H^+ + CN^-$$

The ratio of HCN to cyanide ion is a function of pH. The equilibrium pK for cyanide at 25 C is 9.21 (9); thus, below pH 8, cyanide is largely undissociated.

Although cyanide can hydrolyze or polymerize in aqueous solution, it has been shown that for cyanide concentrations below

650 mg/l and temperatures below 100C, these reactions are insignificant (14). For instance, at 30C and pH 10, the half-life for hydrolysis of cyanide is 459 days and at pH 7, the half-life is 43,000 days. Polymerization is not significant except at high pH, high cyanide concentrations, and high temperatures (14).

Cyanide reacts with aldoses, such as glucose, to form cyanohydrins, which then hydrolyze to the corresponding aldonic acids. This reaction, shown in Figure 1, has been used extensively by carbohydrate chemists for lengthening the aldose carbon chain. The intermediary cyanohydrins usually are not isolated since they are rapidly hydrolyzed in situ (13). The reaction is pH dependent with cyanide ion acting as a nucleophilic reagent. Chemists using this method have used high concentrations of aldoses and cyanide, and no quantitative rate information for the reaction is available. If the reaction occurs at the low concentrations of substrate and cyanide commonly encountered by environmental engineers, and at neutral pH, it could account for a portion of the cyanide removal commonly attributed to biological oxidation and cause errors due to sample storage.

Experimental

Glucose was chosen to investigate cyanide-substrate reactions since it is commonly used in biological investigations and with other aldoses is common in raw sewage and complex media. Using fresh glucose and cyanide solutions and inorganic buffers, kinetics of the glucose-cyanide reaction was studied in sealed glass ampoules to prevent cyanide loss due to volatilization. The reaction was studied at 30C, and the results are shown in Figure 2. Cyanide

was analyzed using the modified Liebig titration method with a precision of ± 0.4 mg/l cyanide as KCN (1).

Results and Discussion

The reaction is pseudo-first order, with an optimum pH of 11.0. Figure 2 indicates that the reaction of cyanide with aldoses can significantly reduce the amount of free cyanide in solution at pH greater than 8.0. The reaction can present problems in storage of certain cyanide-containing samples, but it also offers a possible method for detoxifying cyanide-containing wastes, particularly when a cheap source of carbohydrate is available e.g. wastes from textile mills, canneries, breweries, distilleries, beet-sugar plants, cereal grain processing plants, and pulp and paper mills. A recent patent failed to consider pH requirements, but described batch processes whereby 100 percent cyanide removal was effected in 15 minutes to 4 hours at temperatures from 18 C to 100 C using a starch conversion syrup as a carbohydrate source (10).

BIODEGRADATION OF CYANIDE-ALDOSE REACTION PRODUCTS

Toxicity and biodegradability of cyanide-aldose reaction products are of primary concern to the waste treatment design engineer since the possibility exists for biological oxidation of detoxified wastes.

Experimental

To test for biodegradability of the cyanide-glucose reaction products, three sets of growth experiments were performed. In the first set of experiments, glucose and excess cyanide were reacted for 10 days at pH 11 and the excess cyanide was then stripped from solution. Analyses for residual glucose (15) and cyanide (1)

were both negative. Soluble organic carbon analyses were also run using the Beckman Model 915 analyzer. Twenty ml of the reaction products containing 5.2 mg carbon was added to a 125 ml shake flask, along with 1 ml of inorganic nutrients containing the following components in mg/ml: N, 1.3; P, 0.3; Mg, 0.25; Ca, 0.2; Fe 0.01; and Na, 0.2. The shake flask was then innoculated with 1 ml of unacclimated sewage seed which had been settled overnight at 20 C, blended in a Waring blender for two minutes, and then filtered twice through Whatman No. 2 filter paper to remove protozoa. As a control, a second flask was filled with 20 ml of a glucose solution (5.2 mg total carbon) and 1 ml of the nutrient solution, and then seeded. A third shake flask was filled with 10 ml of reaction product solution, 10 ml of glucose solution, one ml of nutrient solution and one ml of seed. The third flask was to test for possible toxicity of the reaction product. The flasks were stoppered with cotton gauze and shaken at 200 rpm and 30 C in a New Brunswick Psycrotherm incubator. Growth was measured indirectly by measuring absorbance.

A second set of experiments was run identical to the first except that the flasks were innoculated with 1 ml of acclimated seed obtained from the first set of experiments.

A third set of experiments used oxygen uptake to monitor cell growth indirectly. Bottles for manometric measurements (Hach Chemical Co.) were filled with 70 ml of reaction products containing 18.1 mg of carbon, 5 ml of the acclimated seed from the second set of experiments, and 82 ml of dilution water saturated with oxygen. The same ratio of nutrients was used as in the

other experiments. Additional BOD bottles containing the same quantities of carbon (as glucose), nutrients, and seed were used as controls. Two BOD bottles were filled with 5 ml of seed and 152 ml of dilution water containing nutrients.

Results and Discussion

The results of the first set of experiments using unacclimated seed demonstrate that glucose-cyanide reaction products are biodegradable (Figure 3). The results of the second set of experiments indicate that organisms can easily acclimate to the reaction products and that the reaction products are nontoxic to acclimated organisms (Figure 4). Results of the oxygen uptake experiments (Figure 5) further indicate that organisms readily degrade the reaction products. The following equation for balanced growth in a BOD bottle (3) was used to predict oxygen uptake due to conversion of soluble substrate:

$$24C_6H_{12}O_6 + 59O_2 + 17NH_3 = \underset{\text{(bacteria)}}{17C_5H_7NO_2} + 59CO_2 + 110H_2O$$

Based on this stoichiometry, the glucose BOD was expected to be 129. A BOD of 114 was obtained, indicating close agreement. Since aldonic acids contain a higher percentage of oxygen than glucose, a lower BOD was expected and obtained from an equal carbon amount of this substrate.

ADSORPTION ONTO BIOLOGICAL FLOC

Adsorption has been proposed as a means of removing cyanide from waste streams entering sewage treatment plants. A patent has been issued describing cyanide removal by treatment with anaerobic digester solids followed by aerobic biooxidation of the

wastes (8). The anaerobic fermentation sludges are reported to adsorb cyanide, rendering it nontoxic to the subsequent aerobic process. Since the reduced toxicity may occur due to mechanisms other than adsorption, experiments were designed to test the adsorption hypothesis.

Experimental

In the first experiment, a pure culture of a nonflocculating bacterium, Bacillus megaterium, ATCC Strain 2300, was used since this organism is cyanide-resistant and exists as single rods in cyanide-containing media. Uniform morphology made surface area calculations possible. The organism was cultured in tryptic soy broth, centrifuged, and washed four times with physiological saline. Concentrated cells were then drawn into a glass syringe and injected into a 255 ml stirred glass reactor containing a known quantity of cyanide in pH 7.0 phosphate buffer. The reactor was closed with a serological stopper through which solids injection was made. A second syringe was used as a reservoir during solids injection and sample removal. Samples were removed using glass syringes and analyzed for cyanide by the modified Liebig titration method (1).

A second experiment utilized initial aqueous samples from stripping tests conducted in the reactor shown in Figure 6. In these experiments, flocculent heterogeneous cultures were used, since electron micrographs of certain flocculating bacteria have revealed a large polysaccharide matrix which greatly increases the relative number of adsorption sites (6). Since physical adsorption is normally rapid, it was hypothesized that any rapid

loss of cyanide might be attributable to adsorption and would be indicated by unaccounted for cyanide in the material balances during the first 60 minutes of contact time. Starved cells were suspended in a buffered solution containing no substrates, and after adding cyanide, air stripping was initiated.

Results and Discussion

No significant cyanide adsorption occurred in suspensions of nonflocculating cells (Table I). With heterogeneous flocculent suspensions (Table II), rapid initial removal of cyanide occurred which might be attributable to adsorption. Adsorption probably accounted for less than 15 percent of the observed cyanide removal. Thus, although the extracellular composition of organisms does affect adsorption, it plays a minor role in overall cyanide removal in aerobic biological waste treatment.

STRIPPING OF CYANIDE

Very little vapor-liquid equilibrium data exists for aqueous solutions containing low concentrations of cyanide. Since cyanide stripping has been reported to be significant in aerated biological treatment of cyanide-containing wastes (11), this removal mechanism was investigated to determine its relative importance.

Experimental

A New Brunswick Scientific Co. Model MMF-07 Microferm Fermentor was used for control of temperature, air flow rate, and mixing. Humidified, filtered air was used for stripping, and off-gas cyanide and carbon dioxide were captured in 250 ml Fisher-Milligan gas traps filled with 200 ml of 0.1 N NaOH. Heterogeneous flocculent cultures containing no substrate were used, and the

solutions were buffered at pH 7.0 using inorganic phosphate buffers. The reactor system used in these studies is shown in Figure 6. Stripping was investigated at several solids concentrations in a leak tight apparatus.

Results and Discussion

Batch stripping curves for three solids concentrations indicate very little change in stripping rate except for an initial rapid decrease when solids are added (Figure 7). This decrease could be attributable to physical adsorption, metabolism, or even cyanide reaction with biological polymers. Stripping curves for cyanide without solids present (Figure 8), indicate that virtually 100 percent cyanide recovery was possible, but with 3330 mg/l solids present (Figure 9) approximately 17 percent of the cyanide was unaccounted for after six hours, with most of the loss occurring within one hour.

CYANIDE METABOLISM

Since both cyanide adsorption and metabolism were possible causes for less than 100 percent cyanide recovery in the stripping experiments with solids present, cyanide metabolism was studied. Aerobic metabolism of cyanide should be accompanied by production of carbon dioxide, hence, significant amounts of $^{14}CO_2$ produced by bacteria utilizing $K^{14}CN$ should be evidence for metabolism.

Experimental

The same apparatus used in the stripping experiments was used in studying metabolism (Figure 6). Batch cultures of starved heterogeneous flocculent cultures which had been acclimated to cyanide were batch-fed cyanide and glucose. $H^{14}CN$ and $^{14}CO_2$ in

the off-gas were continuously absorbed in washers containing caustic. At the completion of the experiment, washer solutions and the final reactor solutions were analyzed for $H^{14}CN$, $^{14}CO_2$, and ^{14}C incorporated into cellular material. Solids were separated from solution by both filtration through 0.45μ filter paper and by centrifugation. In both instances, the cells were washed several times with physiological saline. Cyanide was separated from carbon dioxide by acid stripping of the caustic samples. Cyanide stripped from the samples was captured in acidified $AgNO_3$; carbon dioxide was trapped in NaOH[2]. Cyanide interference with the Glucostat test for glucose was avoided by stripping cyanide from the samples prior to analysis.

Results and Discussion

Materials balances indicate approximately 95 percent recovery of radioactivity (Table III). Most of the KCN activity was recovered from the off-gas washers, but since the pK of CO_2 is 6.3, a large amount of $^{14}CO_2$ remained in solution. Stripping and metabolism appear to account for approximately 90 percent of total cyanide removal with adsorption playing a relatively minor role, unless subsequent metabolism occurred. The ^{14}C in the cells is either adsorbed cyanide bound so tightly that washing did not remove it, or represents ^{14}C incorporated into cellular material due to metabolism. Glucose was easily metabolized in the presence of cyanide as illustrated in Figure 10. When a primary substrate such as glucose was present, the more active biomass resulted in more rapid cyanide removal than observed in the stripping experiments where no primary substrate

was present.

CONCLUSIONS

1. Significant reactions between cyanide and aldoses such as glucose can occur at hydrogen ion concentrations above 8, indicating that alkaline storage of cyanide samples containing aldoses can result in errors in analysis of cyanide. The reaction with glucose is pseudo-first order, with an optimum pH of 11.0. The reaction products are biodegradeable.

2. In an aerated biological system demonstrating cyanide removal, both cyanide stripping and cyanide metabolism are important removal mechanisms. Adsorption on biological floc is of lesser importance, but the extracellular composition of bacterial cells does influence the degree of cyanide adsorption.

3. In instances where a plant discharging a cyanide-containing waste is in close proximity to a plant discharging an aldose carbohydrate waste, the possibility exists to combine the two waste streams at high pH in a pretreatment step prior to biological oxidation. This pretreatment eliminates HCN as a potential air pollutant and renders the cyanide biodegradable.

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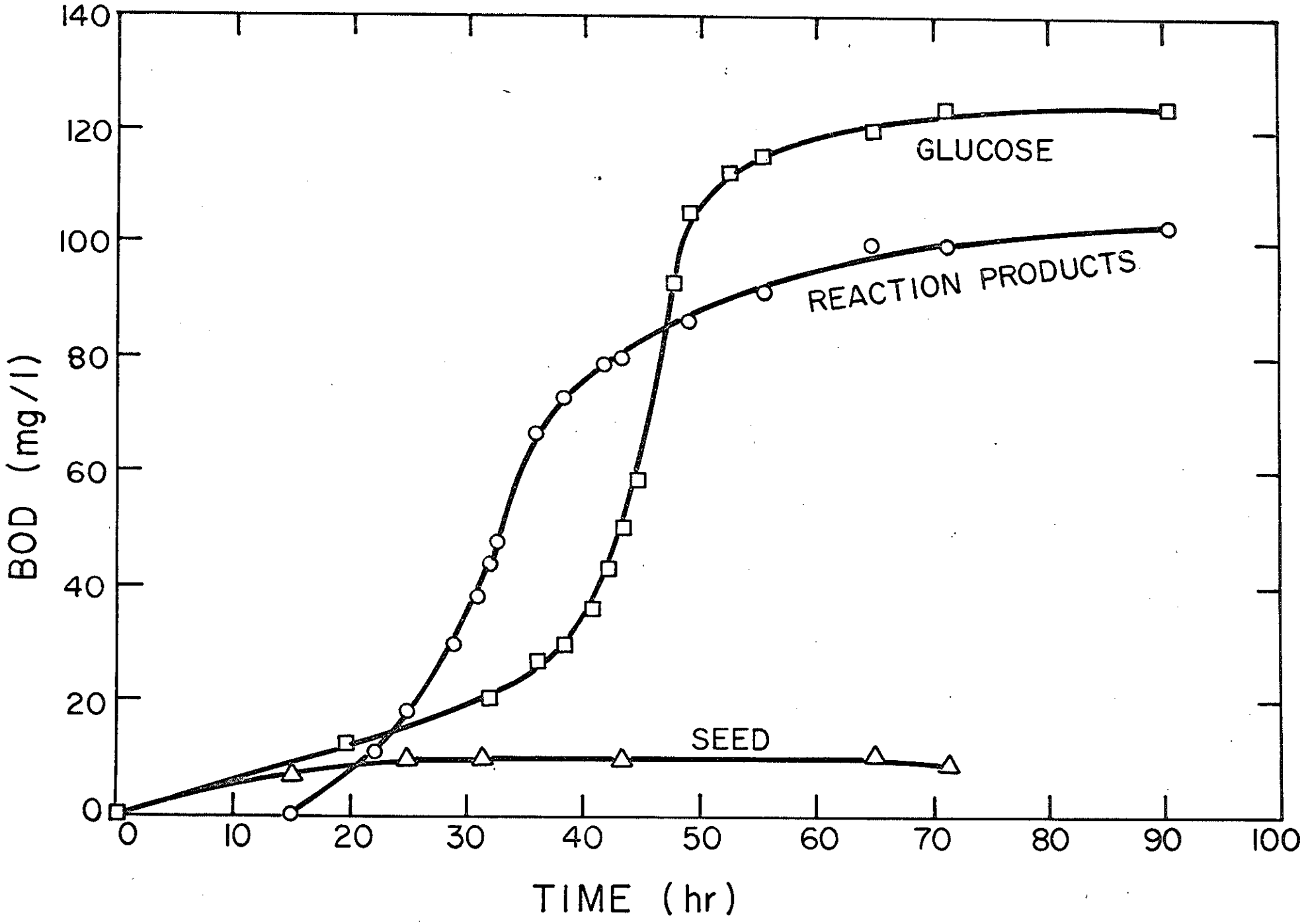
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120
100
80
60
40
20
0
BOD (mg/l)
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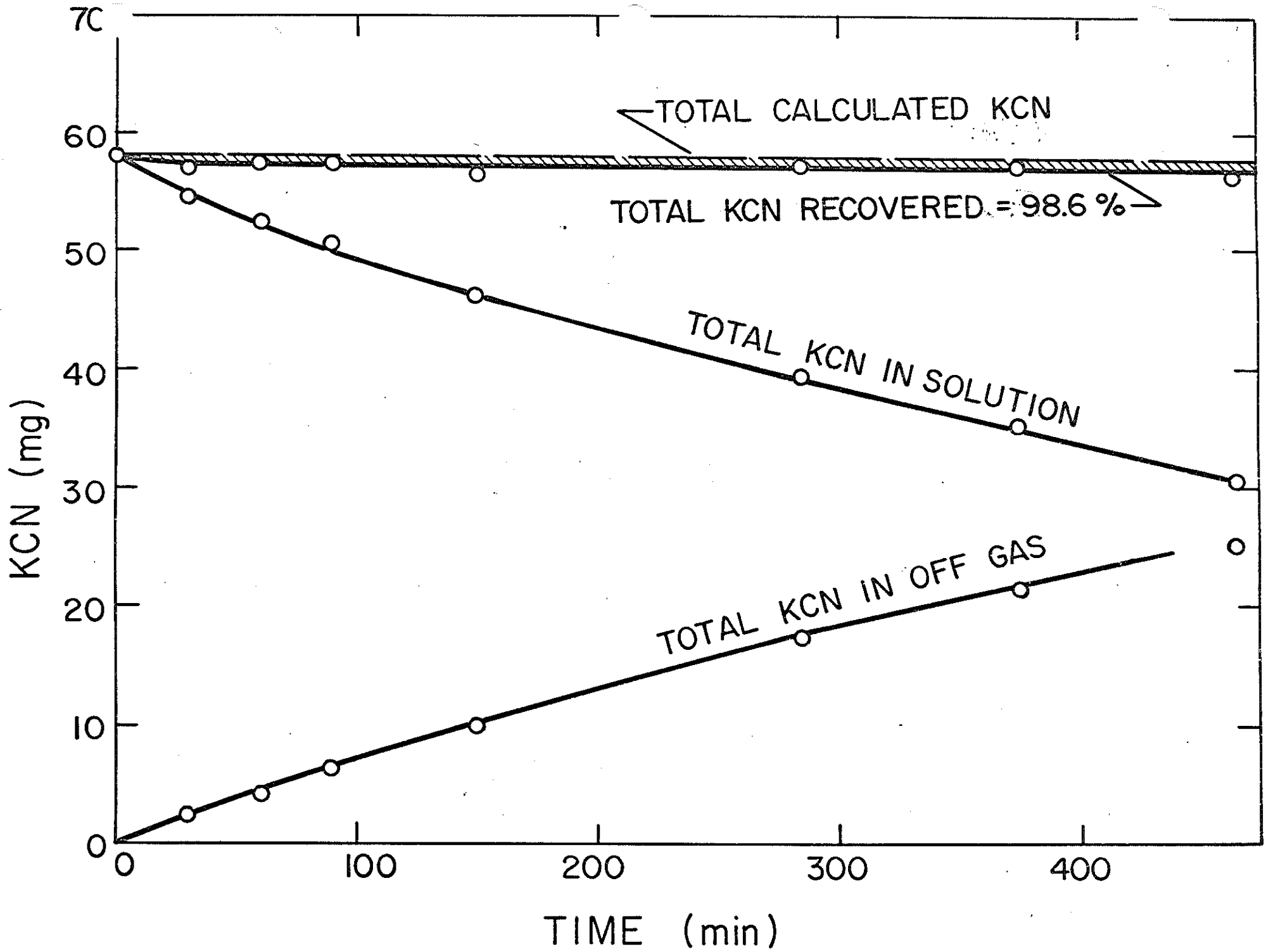
10
20
30
40
50
60
70
80
90
100
TIME (hr)
GLUCOSE
REACTION PRODUCTS
SEED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50
40
30
20
10
0
KCN (mg)
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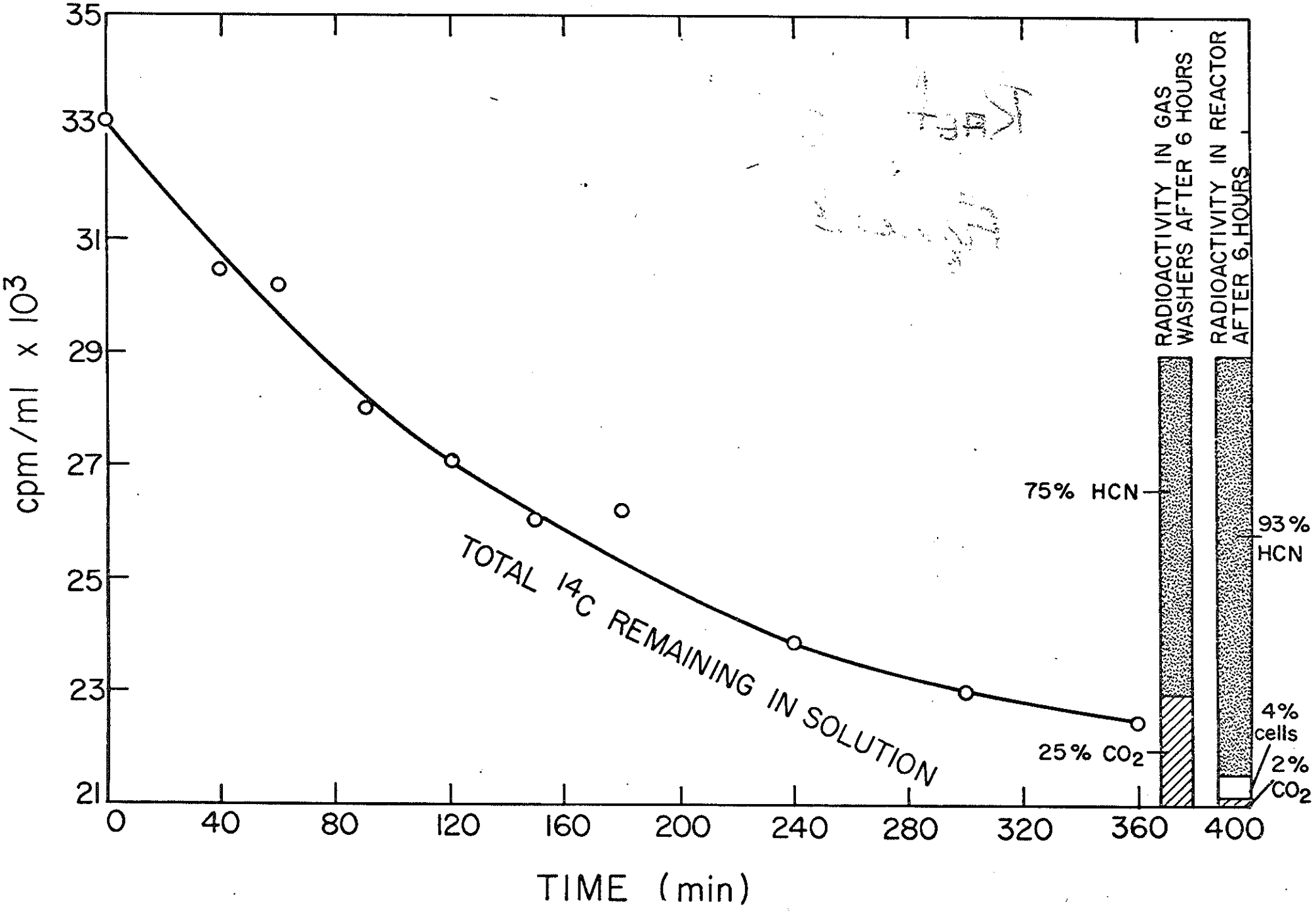
TOTAL KCN IN SOLUTION
TOTAL KCN IN OFF GAS
0
100
200
300
400
TIME (min)


35
33
31
29
27
25
23
21
cpm/ml x 10^3
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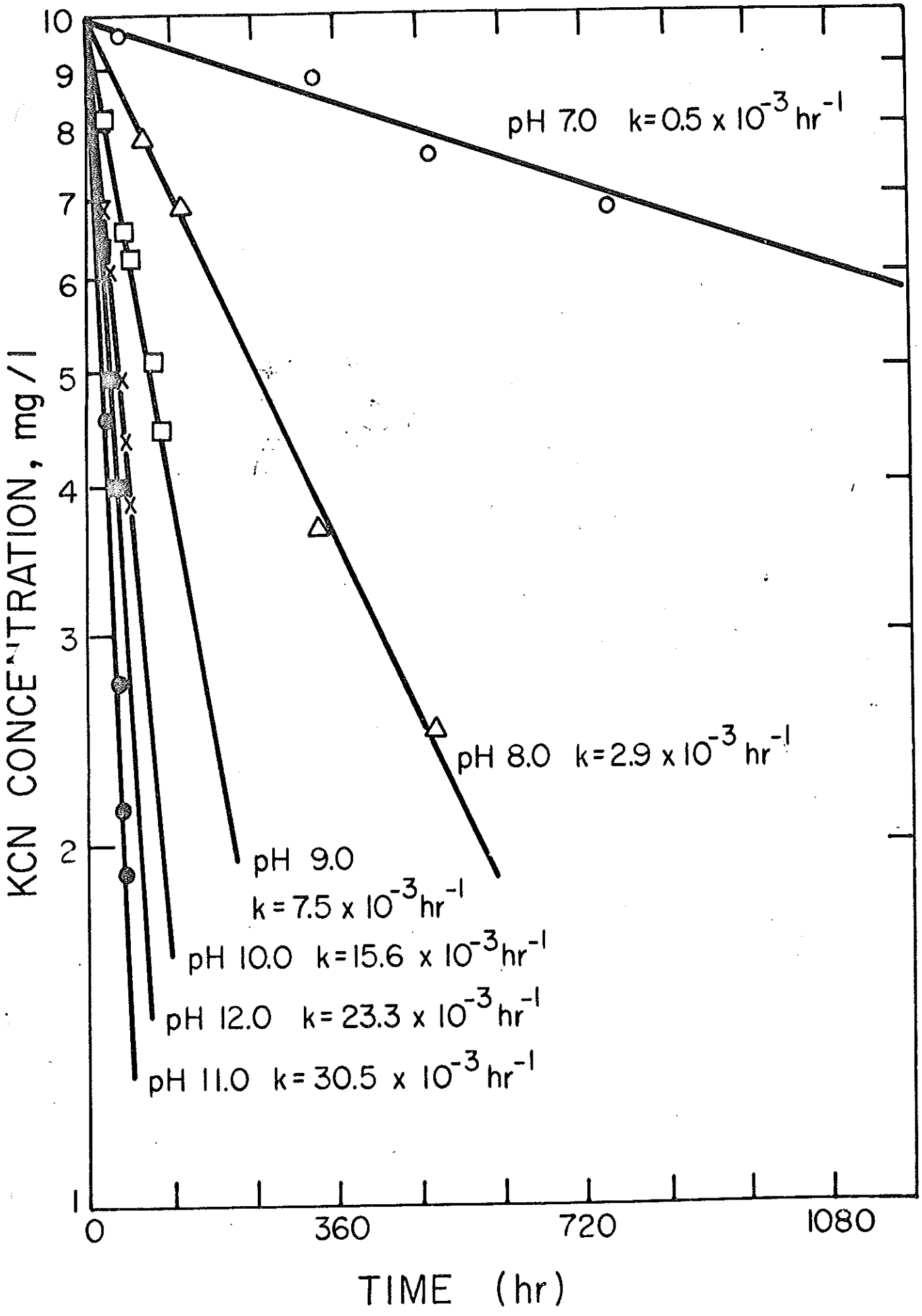
40
80
120
160
200
240
280
320
360
400
TIME (min)
TOTAL ^{14}C REMAINING IN SOLUTION
RADIOACTIVITY IN GAS WASHERS AFTER 6 HOURS
RADIOACTIVITY IN REACTOR AFTER 6 HOURS
75% HCN
93% HCN
4% cells
2% CO_2
25% CO_2


KCN CONCENTRATION, mg/l
TIME (hr)
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pH 8.0 k=2.9 x 10^-3 hr^-1
pH 9.0
k = 7.5 x 10^-3 hr^-1
pH 10.0 k=15.6 x 10^-3 hr^-1
pH 12.0 k=23.3 x 10^-3 hr^-1
pH 11.0 k=30.5 x 10^-3 hr^-1
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9
8
7
6
5
4
3
2
1
0
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720
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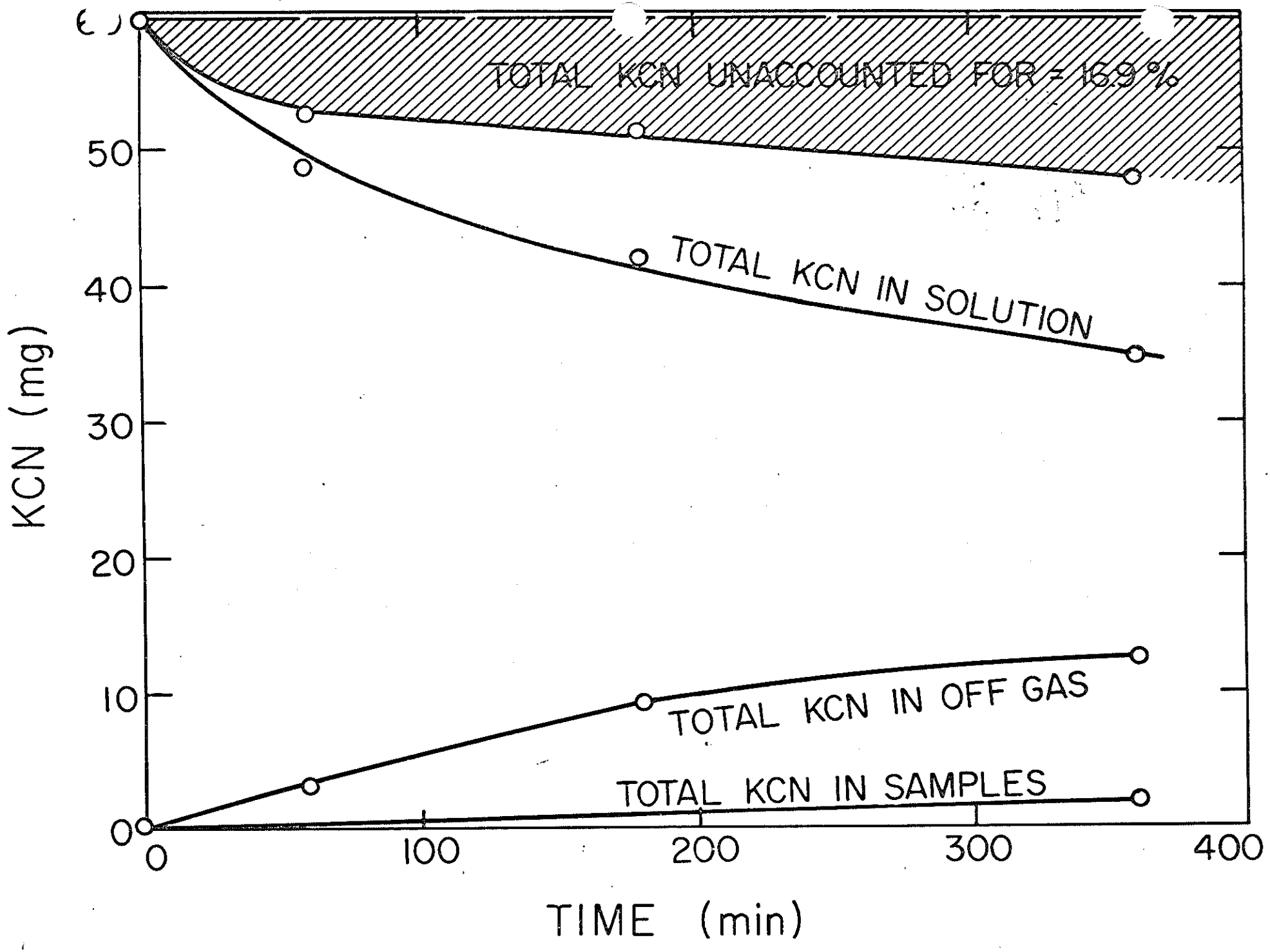
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TOTAL KCN IN SOLUTION
TOTAL KCN IN OFF GAS
TOTAL KCN IN SAMPLES
KCN (mg)
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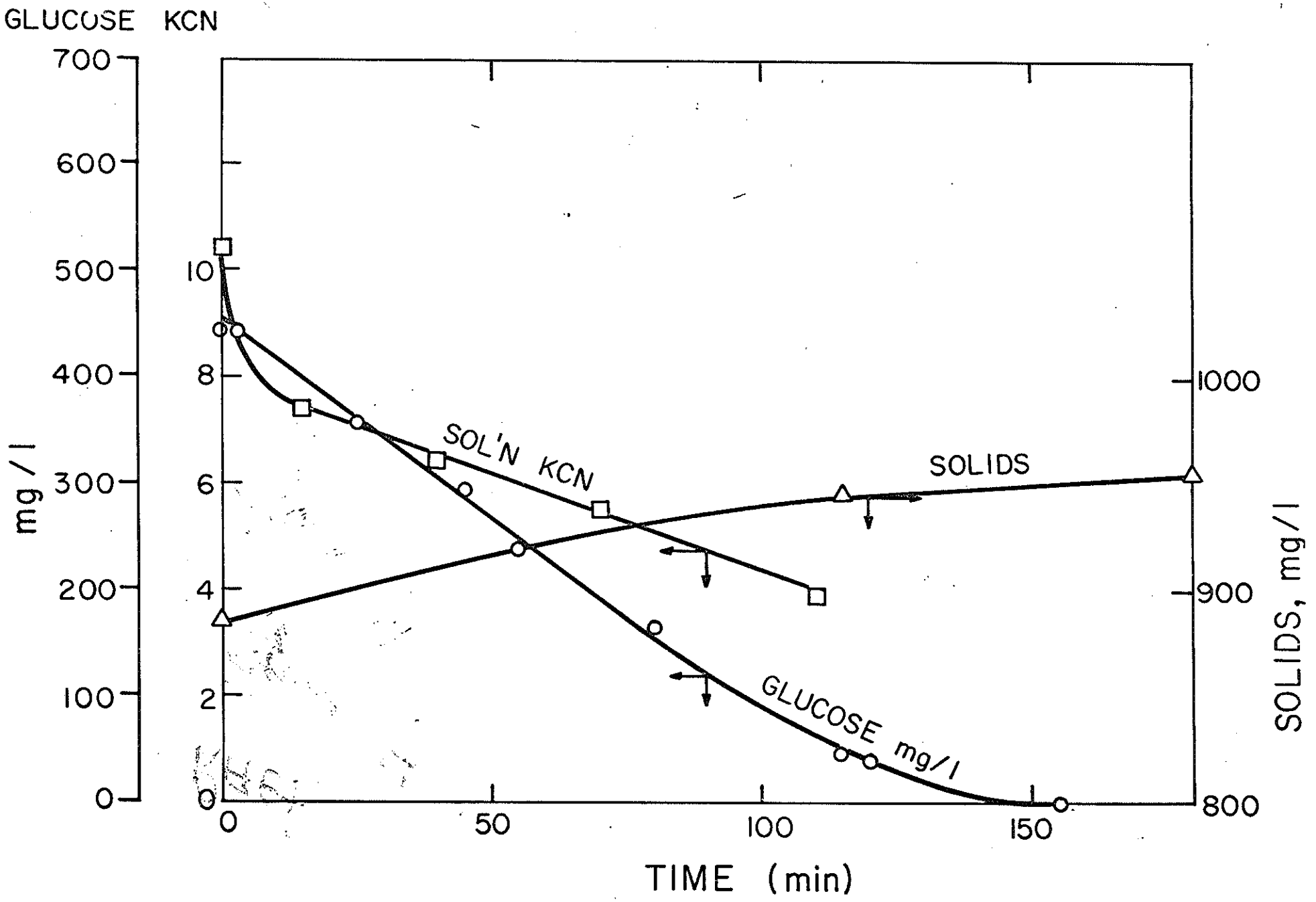
20
30
40
50
0
100
200
300
400
TIME (min)


GLUCOSE KCN
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600
500
400
300
200
100
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mg/l
10
8
6
4
2
0
0
50
100
150
TIME (min)
SOL'N KCN
SOLIDS
GLUCOSE mg/l
1000
900
800
SOLIDS, mg/l