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Improvement of Yields and Rates During Enzymatic Hydrolysis of Cellulose to Glucose

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Abstract

The objective of this program is to show that the conversion of cellulose to glucose can be significantly increased by enzymatically removing the inhibitory cellobiose from the reaction system using immobilized β-glucosidase (β-G). An enzymatic catalyst was prepared and used in a fluidized bed with cellobiose as the substrate, only a 10% loss of activity was observed during a 500 hour period. Cellulose was hydrolyzed in two batch reactors operated side-by-side, with one reactor containing immobilized β-G and cellulose and the other reactor containing an equal amount of cellulose only. After 30 hours the reactor containing the immobilized β-G had 100% more glucose, indicating that the catalytic removal of the cellobiose had a significant effect upon the production of glucose.

Objectives

Since the levels of β-G in the cellulase enzyme mixtures are usually insufficient to prevent the accumulation of cellobiose, this program proposes to add additional β-G immobilized on a suitable support to convert the unwanted cellobiose to desirable glucose. By immobilizing the β-G and allowing its reuse, the glucose cost from the enzymatic hydrolysis process will be reduced.

The immobilized β-G can be used in a variety of reactor configurations. It may be placed into the same reactor as that containing the crude cellulase mixture of enzymes, or it can be operated separately in its own reactor, and connected to the cellulase system by a recycle loop. This program covers the enzyme production, immobilization, and evaluation in reactor systems. Its goals are as follows:

1. Produce microbial β-G from Aspergillis phoenicis.
2. Purify the β-G to see whether immobilization yields are increased.
3. Immobilize the β-G on suitable supports for commercial reactors.
4. Operate the immobilized β-G along with a cellulase reactor to show that yields of glucose are increased by the removal of the inhibitory cellobiose.

During this last quarter we have concentrated on goal numbers 2, 3, and 4.

Experimental Approach:

β-G production and purification

Samples of A. phoenicis from the collection at the University of Massachusetts were obtained and grown in a 14 liter fermentor in a starch medium recommended by Dr. Mandels and Dr. Sternberg of the U. S. Army Natick Laboratories. The culture was grown at 28-30°C for 14 days during which time the pH was adjusted to prevent it from dropping below 4.0. If the pH dropped below 4.0 the yield of β-G decreased.

After 10-14 days the broth was concentrated by ultrafiltration to 1/10 of the original volume using a 30,000 MW membrane. At this point the solution was either freeze dried or further purified by acetone precipitation to give a dry powder.

The acetone precipitated material was compared with the crude freeze dried material to see whether the enzyme loading (EU/gm of support) and the immobilization yield (EU actively adsorbed/EU removed from contacting solution) are significantly increased by the purification. The goal would be to use as impure material as practical to save on purification costs.

Immobilize the β-G on suitable supports.

The initial screening included selecting a suitable support and investigating both the extent of loading possible on the supports and the effect on the activity of crosslinking with a 0.25% glutaraldehyde solution.

One important performance criterion is the life of the immobilized enzyme under reaction conditions. After a suitable support had been chosen based upon loading and activity retention tests, it was placed in a fluidized bed and tested for several days at 50°C using the apparatus shown in Figure 1. A cellobiose solution was used as the substrate and was recycled through the fluidized bed to allow a sufficiently high liquid velocity for fluidization, while still giving a long enough residence time to offer a high conversion. Fresh cellobiose solution was continuously fed into the recycle loop at the same rate as the loop solution was withdrawn, allowing the system to operate continuously at steady state. The glucose concentration was periodically measured in the effluent stream to determine the loss of enzyme activity with time.

Operation of a cellulase reactor with immobilized β-G.

The main objective of this program is to test the immobilized β-G in a reaction system containing cellulase and using cellulose as a feed material.

Two batch reactors of 1.0 liters were run in parallel, one without the immobilized β-G and the other containing it. The initial cellulose concentration was 55 gm/ℓ Solka-floc. The cellulase enzyme concentration was at 225 EU/ℓ. The temperature was held at 50°C during the reaction. 5.0 gms of alumina containing 40 EU/gm of β-G were added to the one reactor. Samples were periodically withdrawn from the reactor and analyzed on a liquid chromatograph for cellobiose and glucose concentrations.

Results

β-G production and purification

Broth concentrations of β-G after about 10 days of fermentation, were found to be > 3.0 EU/mℓ, provided the pH during the fermentation did not drop below 4.0. After filtration to remove the cells and ultrafiltration to concentrate the enzyme, the solution was freeze dried to yield a crude powder with an activity of 0.9 EU/mg. This material was further purified using acetone precipitation to yield a powder having an activity of 16 EU/mg. Both enzyme preparations were immobilized and evaluated.

Immobilization

The supports which were tested together with the percentage enzyme activity retention are given in Table 1. Activity retention is defined as the measured activity of the solid/(original activity of the contacting solution less the residual activity of contacting solution). Thus, for example, 42% of the activity lost during adsorption from the contacting solution was found on the support No. 1. The TiO_2 support (#4 and #7) was found to break up easily under agitation, and therefore was not considered further. The SiO_2 and the DP-1 ion exchange resin showed no immobilized activity as a result of our simple preliminary procedure. One possible reason for this result may reside in the relationship of immobilization pH to the isoelectric point of the enzyme. The best

support was the alumina (Type 7881-A) from Corning. This material did not break up under fluidization and still gives a high activity recovery on immobilization.

Since the alumina (support 1) showed good initial results, it was tested further to explore increased enzyme loading and associated retention. For the loading tests, 5 gms of alumina were always contacted with 50 mℓ of enzyme solution containing different amounts of powdered enzyme. The results for the crude freeze dried powder are given in Figure 2, and for the purified powder are given in Figure 3. The effect of glutaraldehyde cross linking was examined in both cases. For the freeze dried powder, the number of EU adsorbed upon the alumina went through a maximum at a solution concentration of about 175 EU/50 mℓ. Cross-linking with glutaraldehyde appeared to have little effect on both the number of EU adsorbed and the percent activity retention during immobilization. The percent activity retention remained relatively constant at about 50%.

When the purified enzyme powder was used, the EU adsorbed upon the support continuously increased as the solution concentration increased. Final solid enzyme levels were about 375 EU/5 gm of alumina, twice the maximum level reached using the crude freeze dried powder. As the amount adsorbed upon the alumina increased, the percent activity retention decreased, although the range still remained between 40 and 50%. Glutaraldehyde cross linking reduced both the enzyme loading and the activity retention of the purified enzyme. The purified enzyme offers sufficiently higher support loadings to make it worth considering for the final reactor design.

The decrease of the enzyme activity with time while converting cellobiose to glucose is given in Figures 4 for no crosslinking and in Figure 5 for glutaraldehyde crosslinking. Glutaraldehyde does not change the active life of the supported enzyme. Both cases had activity losses of about 10% in 500 hours of continuous operation, and both cases used the crude freeze dried enzyme as source

material. This supported enzyme is quite stable and offers a practical support for further studies. The active life of the purified material will be investigated also.

Immobilized β-G and Cellulase Reactor

The results from running the batch cellulase reactor both with and without the addition of immobilized β-G addition are shown in Figure 6. For the case of no β-G addition the cellobiose and glucose concentrations continuously increased, such that at 30 hours, their concentrations were approximately equal. With the addition of the immobilized β-G, the glucose concentration increased by approximately 100% from the conversion of the cellobiose. There was a low residual cellobiose concentration which remained constant at about 0.3 gm/ℓ. The Solka-floc does not make all of its cellulose accessible to the cellulase enzymes because of its crystallinity. Other practical cellulose materials with higher accessibility, such as pretreated corn stover and poplar wood, will be tried. The addition of immobilized β-G to the cellulase mixture dramatically increases the yield of glucose at the expense of the undesirable cellobiose.

Future Plans

The following is a description of the experimental work we plan to undertake during the remainder of this research project.

Feedstocks (Substrates)

Initial experiments have been aimed at learning technical feasibility, and at developing new methods and techniques to immobilize the enzyme for several reactor configurations. Most of this initial work was done with rather pure and refined forms of cellulose for the sake of reproducibility and in order to have a basis for comparing the results with related work of others. Plans are underway to obtain samples of other less refined cellulosic feedstocks and it is expected that it will be possible to use some of these materials in some initial trial experiments towards the end of the first year of this project.

Figure Titles

Figure 1. Fluidized Bed Apparatus for Evaluating Immobilized Enzyme Half-Life.

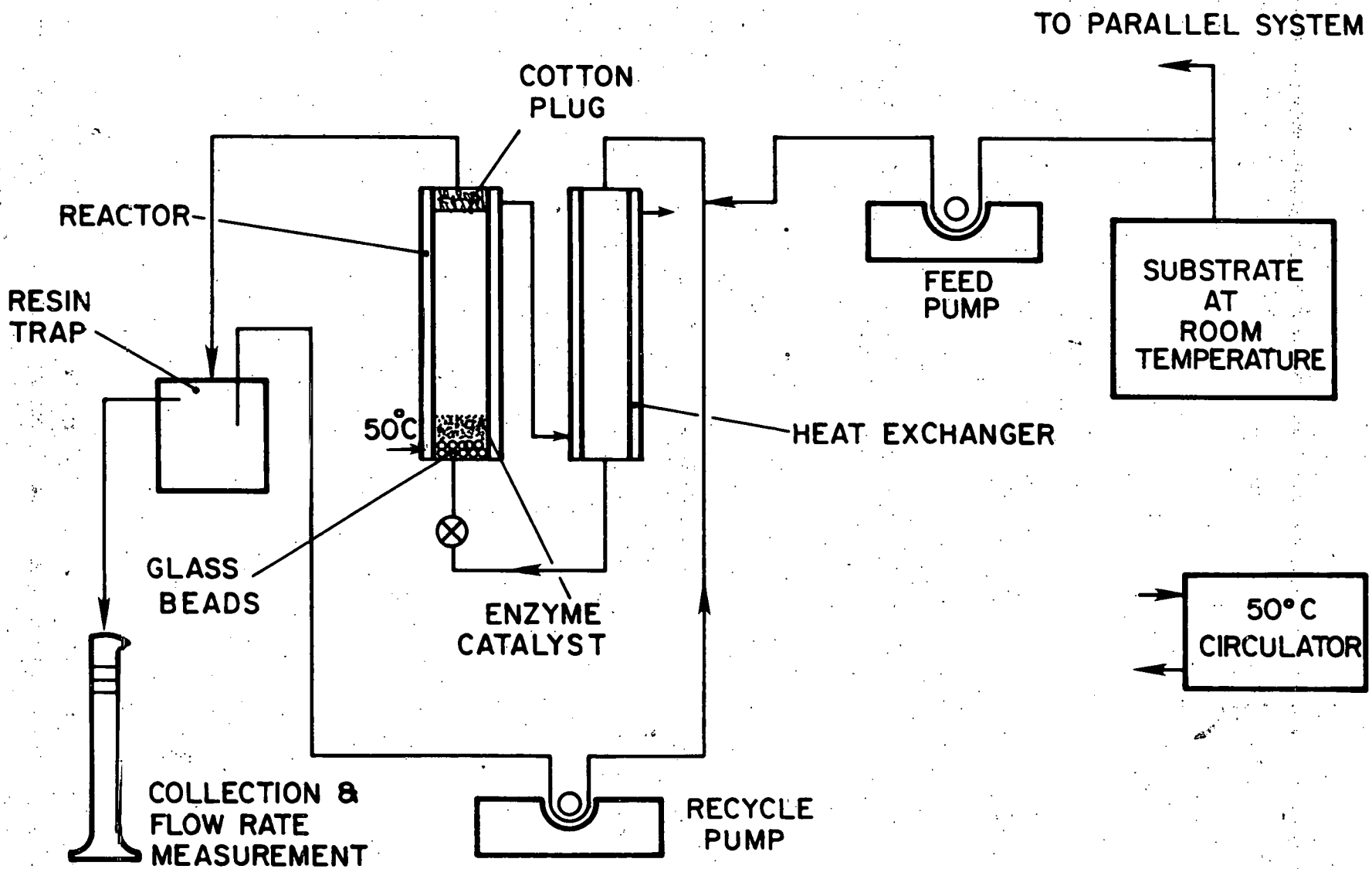
Figure 2. Solid Loading and Activity Retention of 5 gms of Alumina when Exposed to 50 mℓ of Buffer Containing Various Amounts of Crude Enzume.

Figure 3. Solid Loading and Activity Retention of 5 gms of Alumina when Exposed to 50 mℓ of Buffer Containing Various Amounts of Purified Enzyme.

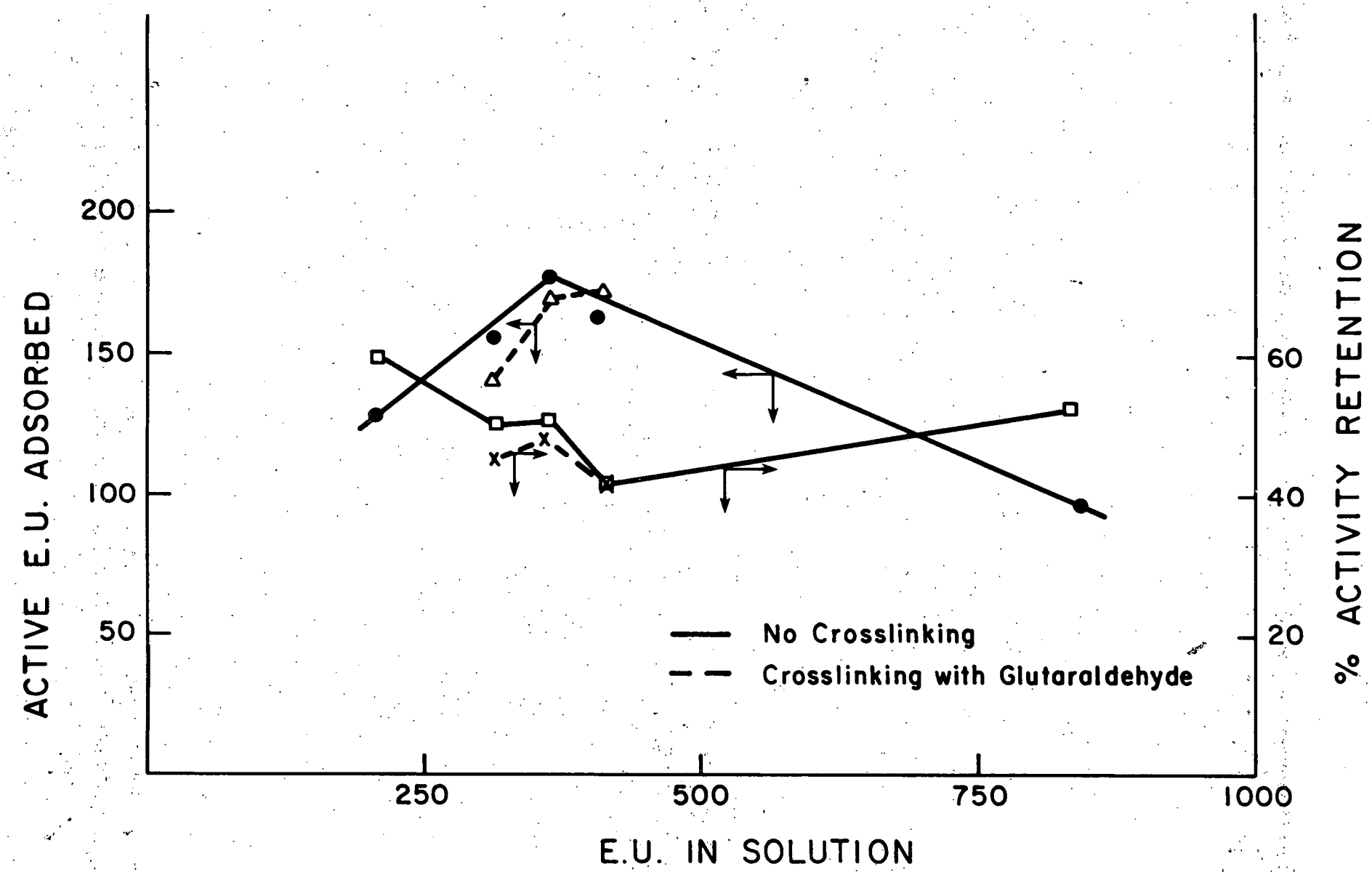
Figure 4. Immobilized Activity versus Reaction Time with Cellobiose for No Crosslinking.

Figure 5. Immobilized Activity versus Reaction Time with Cellobiose for Crosslinking with 0.25% Glutaraldehyde.

Figure 6. Glucose and Cellobiose Concentration in Batch Reactors with/without Addition of Immobilized β-Glucosidase.



FLUIDIZED BED WITH RECYCLE


ACTIVE E.U. ADSORBED
200
150
100
50
250
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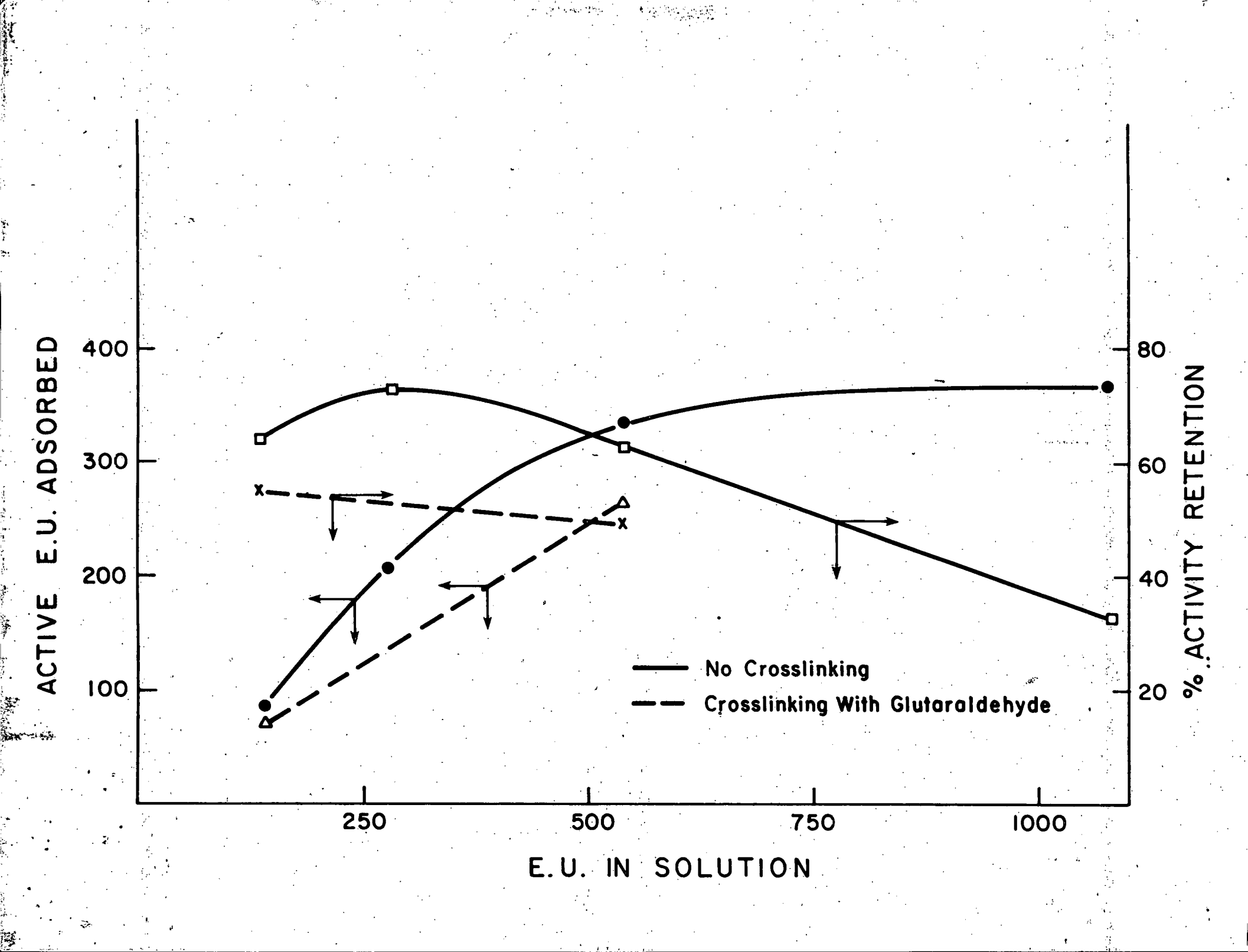
750
1000
E.U. IN SOLUTION
60
40
20
% ACTIVITY RETENTION
No Crosslinking
Crosslinking with Glutaraldehyde


ACTIVE E.U. ADSORBED
400
300
200
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% ACTIVITY RETENTION
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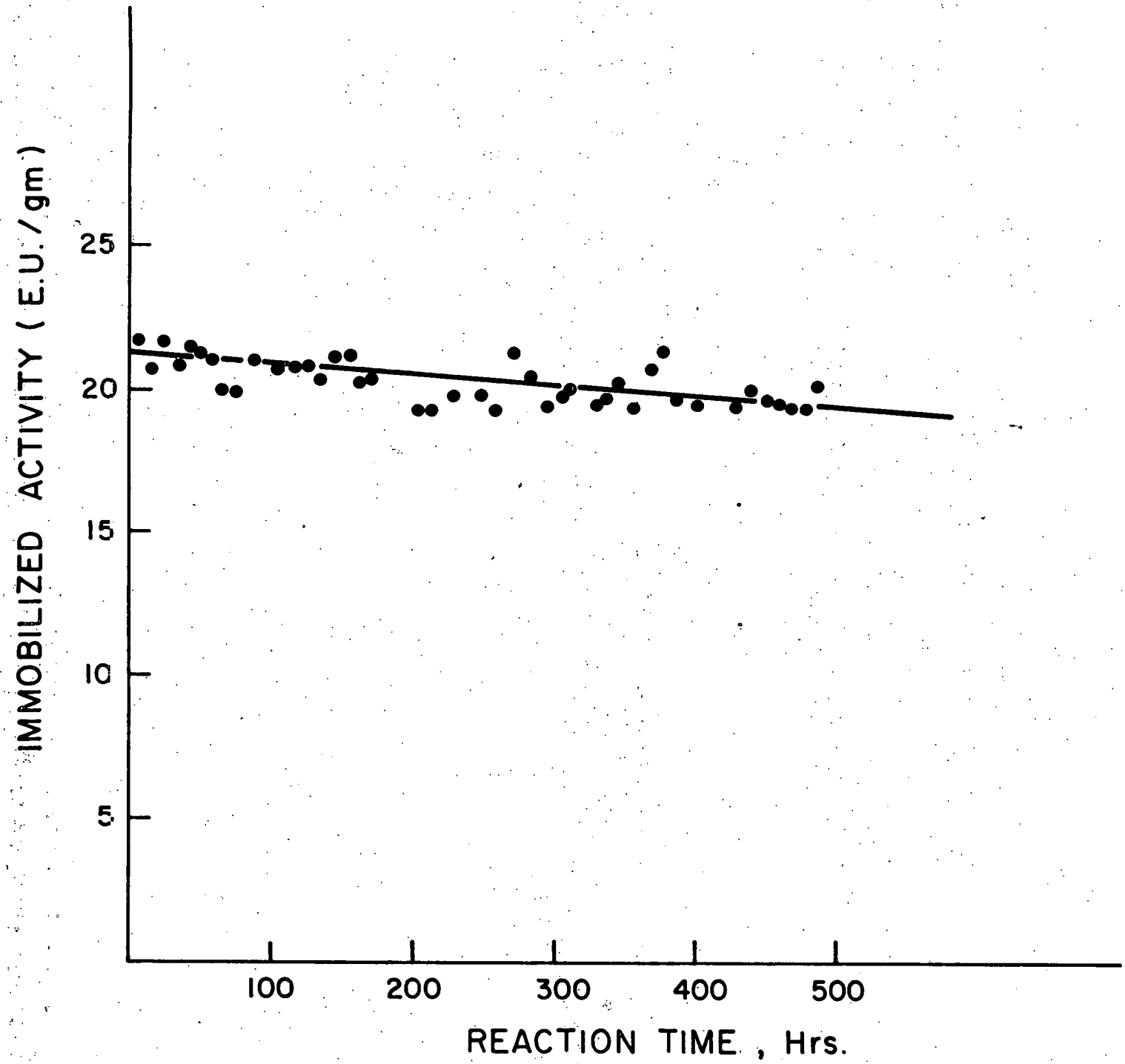
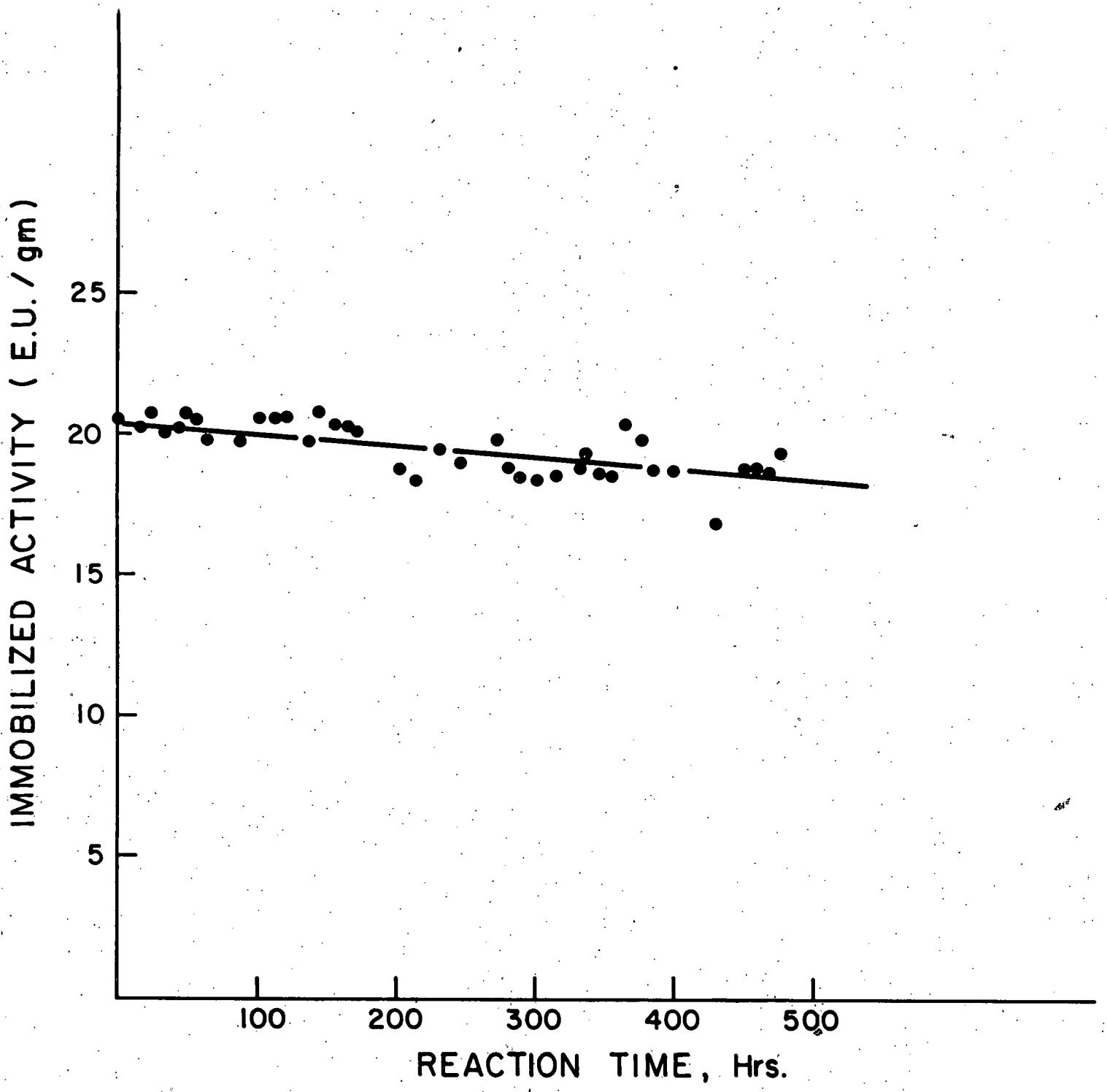
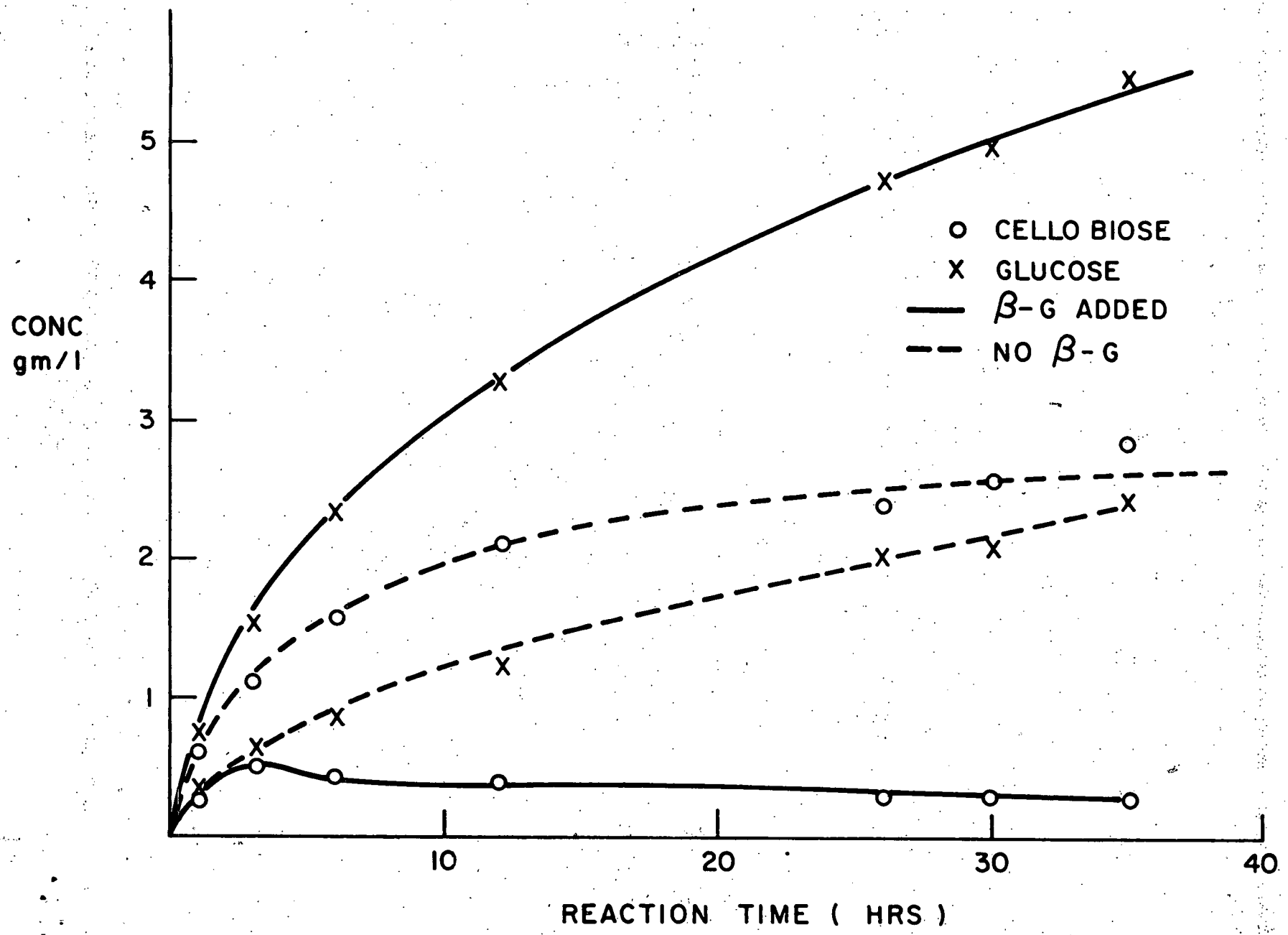
E.U. IN SOLUTION
No Crosslinking
Crosslinking With Glutaraldehyde


IMMOBILIZED ACTIVITY (E.U./gm)
25
20
15
10
5
100
200
300
400
500
REACTION TIME , Hrs.


IMMOBILIZED ACTIVITY (E.U. / gm)
25
20
15
10
5
100
200
300
400
500
REACTION TIME, Hrs.


CONC
gm/l
5
4
3
2
1
10
20
30
40
REACTION TIME (HRS)
O CELLO BIOSE
X GLUCOSE
β-G ADDED
NO β-G