XXI CONGRESS OF THE I.U.L.T.C.S.

COMPENDIUM OF ADVANCED TOPICS ON LEATHER TECHNOLOGY

VOLUME 2

ASOCIACION QUIMICA ESPANOLA
DE LA INDUSTRIA DEL CUERO (A.Q.E.I.C.)

I. S. B. N. : 84 - 604-0107-3
Depósito Legal.: B - 30.519 - 1991

Publisher - Editorial : A. Q. E. I. C.
Avenida de les Corts Catalanes, 608, 8.º C - 08007 BARCELONA (España)

Editor - Coordinador: Jaime Cot Cosp

Impreso por Imprenta (Printer) CARCELLER
Zamora, 95, 4.º, 1.ª - 08018 Barcelona

EFFICIENCY OF ENZYMATIC SOLUBILIZATION OF CHROME SHAVINGS AS INFLUENCED BY CHOICE OF ALKALINITY-INDUCING AGENTS*

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SUMMARY

Enzymatic processing of chrome shavings has been shown to be a viable treatment of this waste product. Proteolytic enzymes, active at moderate temperatures are effective in solubilizing the protein and, since the reaction takes place at an alkaline pH, the chromium remains insoluble. Various alkalinity-inducing agents, such as magnesium oxide, alone or in combination with calcium hydroxide, sodium hydroxide or sodium carbonate, can be employed to maintain the optimal environment for enzyme catalysis. These systems can be tailored to match the varied chrome recycling steps that different tanneries employ. Some of these systems have improved the rate of solubilization of the shavings while lowering the required amount of enzyme, thus making the process more cost effective.

RESUME

La méthode enzymatique de déchromage a démontré être un traitement possible pour ce déchet. Les enzymes protéolitiques, actives à températures moyennes, sont efficaces par solubilisation des protéines et étant donné que la réaction est réalisée à pH alcalin, le chrome reste insoluble. Le dèchromage precedé par des agents induisant l'alcalinité comme préparation pour la dégradation enzymatique. L'oxyde de mangésium, seul ou en combinaison avec l'hydroxyde de calcium, l'hydroxyde de sodium ou la carbonate de sodium a prouvé être hautement efficace dans ce rôle, le pH optimal est maintenu tout au long de la digestion enzymatique. L'ensemble du processus peut être adapté aux divers étapes recyclant le chrome que les différentes tanneries utilisent. Le procédé est conduti à relativement basse température, ce qui non seulement économise de l'énergie mais aussi diminue la possibilité d'hydrolyse des protéines. Les caractéristiques du résidu chromique isolé par ces traitements seront aussi discutées et le potentiel pour le recyclage de ce chrome dans les procédés de tannerie sera décrit.

INTRODUCTION

It has been documented in the literature, including recent reports on work from our laboratory(1-12), that chrome shavings can be treated with enzymes to recover a soluble protein which may have commercial use, and a chromium cake, which has the potential to be recycled by the tanning industry or used in other industrial applications. The methods that were developed at this laboratory have shown that shavings can be subjected to moderate pretreatment temperatures and that the enzyme can be added at these temperatures, thus eliminating the need to cool the reaction mixture, a waste of energy and a time consuming step.

Our preliminary investigations used calcium hydroxide to control the pH(9-12). We were able to achieve 78% solubility of the shavings when 6% of an alkaline proteolytic enzyme was used for hydrolysis. We report here the results of our attempts to achieve higher solubility and at the same time use of lower amounts of enzyme, thus making the treatment more cost effective.

The chemistry of magnesium oxide is similar to that of calcium oxide. It has been documented in the literature that shavings can be treated at 300ºC. and at 150 atm. in the presence of magnesium oxide for the purpose of chromium recovery(13), and hydrolyzed at 100ºC. with sodium hydroxide, when magnesium oxide or calcium oxide are present(14). This paper will describe the results of our experiments using magnesium oxide at more moderate temperatures to achieve the highest solubility with lower amounts of enzyme than previously reported. Also, since precipitation of the chromium, in an industrial recycling system, can be carried out using magnesium oxide as well as a variety of other alkaline agents, we will show the results of our experiments using these compounds with enzyme and the improvement in solubility that results when these other agents are used in conjunction with magnesium oxide.

EXPERIMENTAL

Materials

Chrome shavings were obtained from a commercial tannery and were stored at 4ºC. until use.

Alcalase® (alkaline protease) was obtained from Novo Nordisk Bioindustrials, Inc. (Danbury, CT). It is a proteolytic enzyme with optimal activity at pH 8.3-9.0 and a temperature of 55-65ºC. It is supplied both as a granular solid (adsorbed onto an inert carrier), which is standardized to contain 2.0 AU/g (Anson Units/g), and as a liquid, which is standardized to contain 2.5 AU/g.

Magnesium oxide was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and from Martin Marietta Magnesia Specialties (Hunt Valley, MD) as MagChem 50®.

Procedure

The chrome shavings were suspended in 500% float. Zero to 6% magnesium oxide alone, or in combination with calcium hydroxide, sodium hydroxide or sodium carbonate, was added. The samples were shaken in a Fisher Versa-Bath®S, model224, and heated at 60.5ºC. for 30 min. Zero to 6% solid Alcalase® was added and the samples were further incubated for 160 min. The samples were filtered under vacuum through Whatman #1 filter paper, using porcelain funnels. The residue was weighed after being dried in a gravity oven at 50ºC. for 19 hr. The percent residue was calculated based on the dry weight of the shavings (measured separately). Part of the magnesium oxide and lime remained insoluble throughout the treatment. Inert enzyme carrier and the insoluble magnesium oxide and lime contributed to the residue and controls were run to correct the residue weight.

RESULTS AND DISCUSSION

Preliminary experiments on the enzymatic treatment of chrome shavings used calcium hydroxide for pH adjustment. In these experiments, the initial pH of the reaction, or the holding pH, was about 10.5. As the reaction proceeded, the pH fell to about 8.3. These reactions were carried out at temperatures which ranged from 55 to 75ºC., with optimal reaction temperature at about 60-63ºC. It was found that under these reaction conditions, 6% enzyme was needed to achieve the highest solubility, about 78%. We felt that the solubility could be improved and at the same time, the amount of enzyme could be reduced. The following experiments describe how this goal was attained.

Magnesium oxide is similar in its chemistry to calcium oxide and calcium hydroxide. It has a very low solubility in water at 25ºC., but differs from lime in that the solubility increases slightly with an increase in temperature. Preliminary experiments with magnesium oxide showed that it could possibly be used for pH adjustment of chrome shavings prior to enzyme hydrolysis.

In the first set of experiments, from 0 to 6% magnesium oxide was added to the shavings. As seen in Figure 1, the initial pH's ranged from 3.3 to 9.2 and the final pH's ranged from 3.8 to 8.5. Five to six percent magnesium oxide provided the optimal pH range for the enzymic hydrolysis to take place.

The main objective of this research was to make the treatment more cost effective by reducing the amount of enzyme needed, at the same time retaining or improving the efficiency of the reaction. As seen in Figure 2, 0 to 5% enzyme was added in the presence of 5% magnesium oxide. Eighty percent solubility was achieved when only 1% enzyme was used. Solubility decreased little as concentration of enzyme was increased above1%.

We have found that multiple feeds of enzyme improve the efficiency of the reaction due to the fact that the activity of the enzyme falls off quite rapidly at the temperatures at which we are running these reactions. A total of 1 to 2% enzyme added in multiple feeds is as effective in solubilization as a single feed using 6% enzyme. This indicates that the larger amount of enzyme is being wasted because the activity is being reduced by the high temperatures.

Magnesium oxide is more expensive than lime. We felt that by running combinations of lime and magnesium oxide we could optimize on the effect of the magnesium ion while at the same time utilizing the higher alkalinity provided by the lime.

In the next set of experiments, we used from 0 to 6% lime in combination with 0 to 5% magnesium oxide. Six percent Alcalase® was used with all combinations. Figure 3 gives the solubilities of chrome shavings when the shown combinations were used for pH adjustment. Correlation studies were run to see what effect final pH, magnesium oxide and/or lime have on solubility. The following correlation coefficients, r, were calculated, where p = probability and n=number of observations:

Final pH ($r = 0.876$, $p < 0.0001$) $n = 42$
MgO ($r = 0.762$, $p < 0.0001$) $n = 42$
Lime ($r = 0.45$, $p < 0.003$) $n = 42$

It appears that final pH and magnesium oxide have a high correlation with solubility. When lime is used the correlation with solubility is lower.

Those magnesium oxide-calcium hydroxide combinations from the previous study which gave the highest solubility were subjected to varying enzyme concentrations. Again, as seen in Figure 4, there is a marked improvement in solubility, even when the lower concentrations of enzyme are used.

Sodium hydroxide and sodium carbonate are two of several chemicals that are used to precipitate chromium when chromium recycling is used in the tannery. It would be of benefit to the industry if the pH adjusting agent were similar to that used in precipitation. In the following experiments, these two compounds were used alone and with magnesium oxide to see what effect they would have on the solubility of chrome shavings.

In the first set of experiments, from 1 to 5% sodium hydroxide, in the presence of 1% magnesium oxide, was used to adjust the pH of the shavings. Six percent Alcalase® was used for hydrolysis and the temperature was maintained at 60.5ºC. The magnesium oxide was added first and the reaction was run for 30 min. Then the sodium hydroxide was added and the reaction was again run for 30 min. The holding pH was measured and then the enzyme was added. Figure 5 gives the results of these experiments. The insert shows the effect of the sodium hydroxide concentration on the pH. The initial pH's for the first three concentrations gave a pH range that was too low for optimal enzyme activity. The 5% concentration gave an initial pH of about 10.8, and this is dangerously close to inactivating the enzyme. Four percent sodium hydroxide provided the optimal pH range for the enzyme.

In the next set of experiments, from 4 to 10% sodium carbonate was used, in conjunction with 1% magnesium oxide, to adjust pH. Again, the magnesium oxide was added first, and after 30 min the sodium carbonate was added. Six percent Alcalase® was used for hydrolysis. Figure 6 shows the results of these experiments. The insert shows the pH range for these sodium carbonate combinations. The lower concentrations gave initial pH's that ranged from 8.2 to 9.2 and final pH's that ranged from 7.9 to 8.3 and were too low for optimal enzyme activity. Nine to ten percent sodium carbonate provided an optimal pH environment for the enzyme.

We were interested in the amount of magnesium oxide that would be needed to give the highest solubility with the optimal amounts of sodium hydroxide and sodium carbonate. In this set of experiments, from 0 to 2% magnesium oxide was added to the reaction mixture. Figure 7 shows the results of these experiments. It appears that the 1.5% concentration of magnesium oxide should give optimal solubility of about 84% when used in conjunction with sodium hydroxide and sodium carbonate. The values shown in Figure 7 also indicate that in the absence of magnesium oxide we achieved 70% solubility with sodium hydroxide and 77% solubility with sodium carbonate. In our previous work with lime, no matter how we altered conditions, we could not achieve higher than 78% solubility. It appears that the presence of magnesium oxide helped to achieve the high solubilities reported above.

The final set of experiments addressed the minimum amount of enzyme that would be needed with the optimal amounts of sodium hydroxide and sodium carbonate in the presence of magnesium oxide. As seen in Figure 8, 3% enzyme was needed to achieve 84% solubility when sodium hydroxide was used. The insert shows that, when the 1 and 2% additions were used, the pH did not drop and the enzyme was probably inactivated. Possibly, when using sodium hydroxide, magnesium oxide in higher than 1% amounts should be used with less sodium hydroxide to obtain pH control. Figure 9 shows that 1% enzyme, in the presence of sodium carbonate and magnesium oxide, will give 81% solubility and the insert shows that the pH range is optimal for the enzyme.

Magnesium oxide gives greater solubility under the conditions specified than does lime. Observation of the shavings during the holding period, that time when the pH and temperature are adjusted to optimal for the substrate being treated, shows that they are being totally disrupted, to the point that it appears that hydrolysis has begun. This is not so, for filtration and subsequent drying of the residue do not show any increase in solubility over a control. However, when the enzyme is added, one can observe an almost immediate reaction, the solution may foam and the yellow protein color begins to show.

The above conditions and observations apply only to chrome shavings and not to tear-offs or pieces of blue stock, which may be mixed in with the shavings. Nor do these conditions apply to shavings from blue stock that may have had extraordinary treatment prior to tanning or have had a tanning step that might have introduced extra crosslinks to the collagen. These types of substrates will necessitate an adjustment in the treatment and will be the topic of a future publication.

CONCLUSION

Various alkalinity-inducing agents can be used to adjust pH to optimal for enzymatic hydrolysis of chrome shavings. It has been shown that magnesium oxide, alone or in combination with calcium hydroxide, sodium hydroxide or sodium carbonate, will increase the efficiency of the solubilization and, at the same time, reduce the amount of enzyme needed, thus making the treatment more cost effective. These various alkalinity-inducing agents will enable the tanneries to tailor the process to their individual chromium recycling step. The present findings are the subject of a patent application.

ACKNOWLEDGEMENT

The authors would like to acknowledge **Dr. John G. Phillips** for his correlation analysis of the data.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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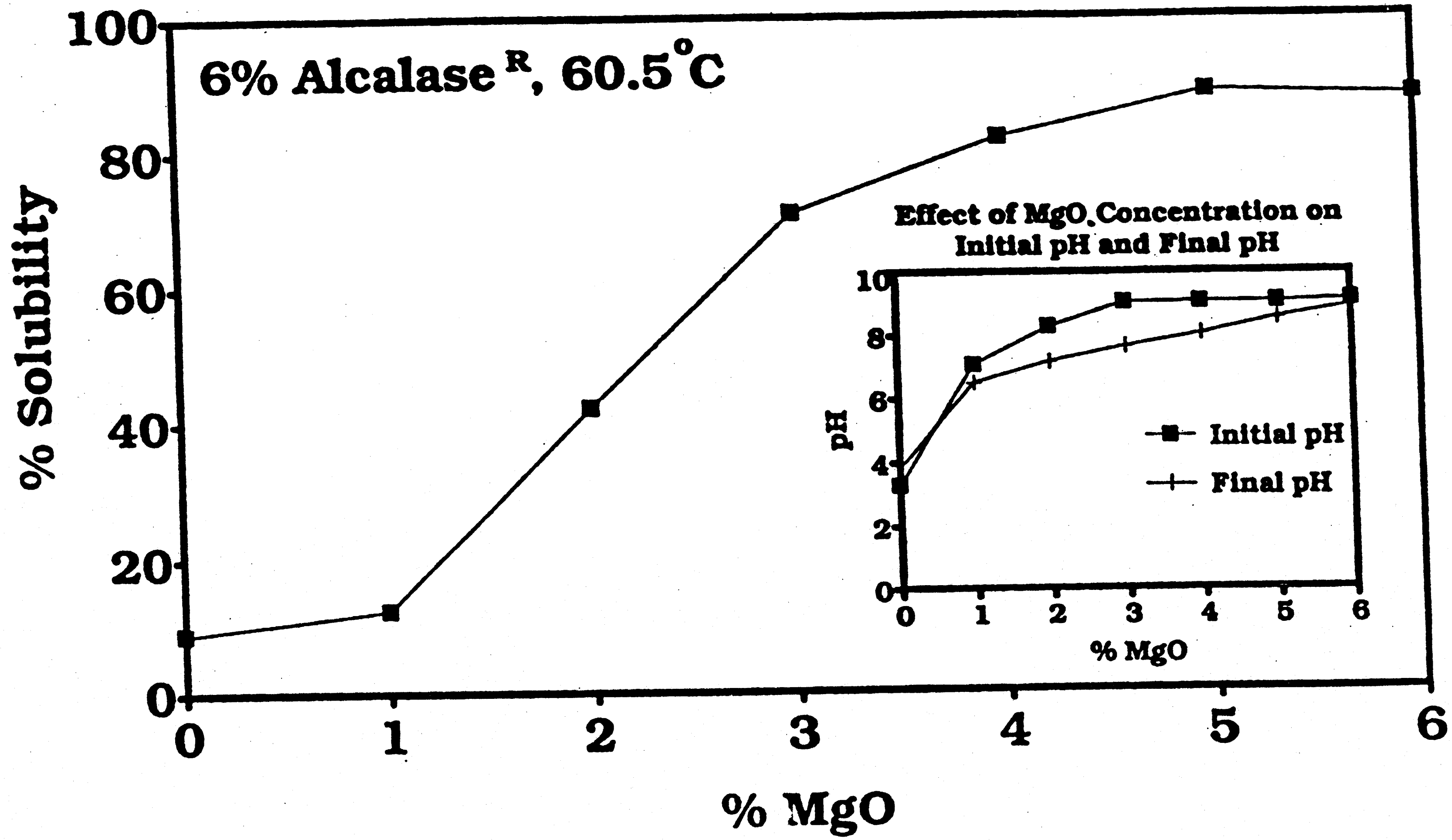


Effect of magnesium oxide concentration, expressed as percent of wet weight, on the solubility of chrome shavings.

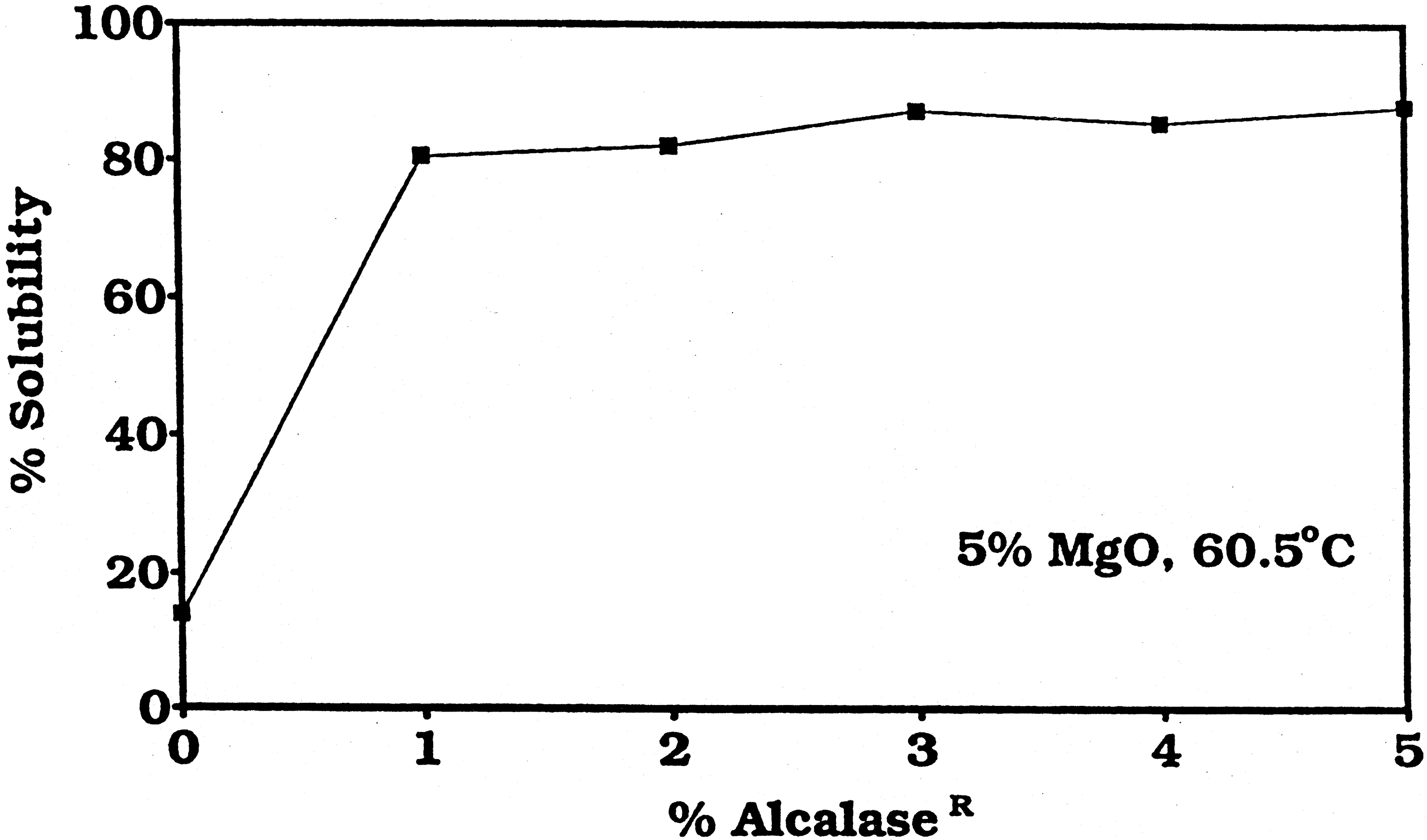


Figure 2. Effect of Alcalase® concentration, expressed as percent of wet weight, on the solubility of chrome shavings.

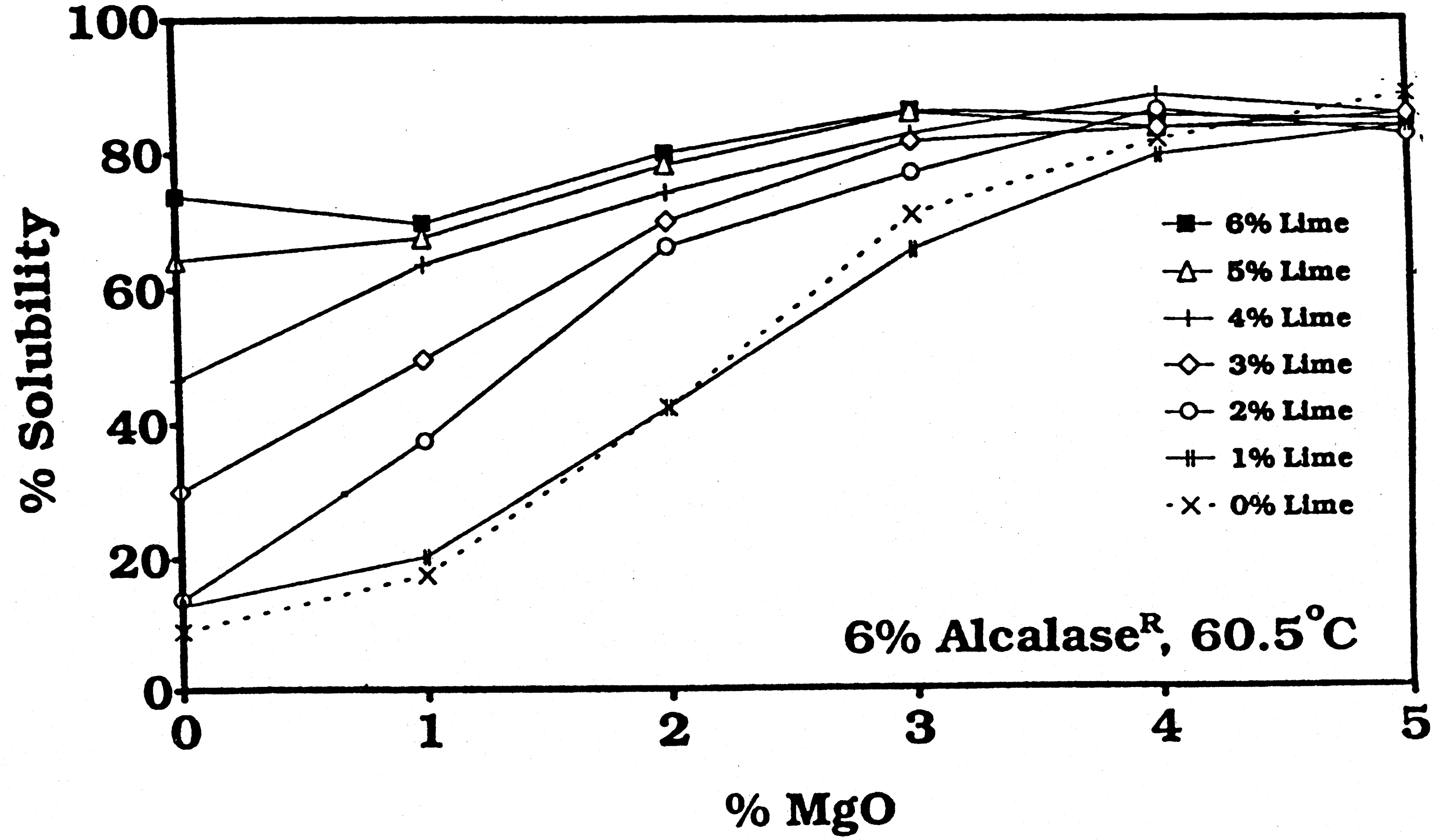


Figure 3. Effect of varying magnesium oxide and lime concentrations, expressed as percent of wet weight, on the solubility of chrome shavings and on the initial and final pH's of the treatment.

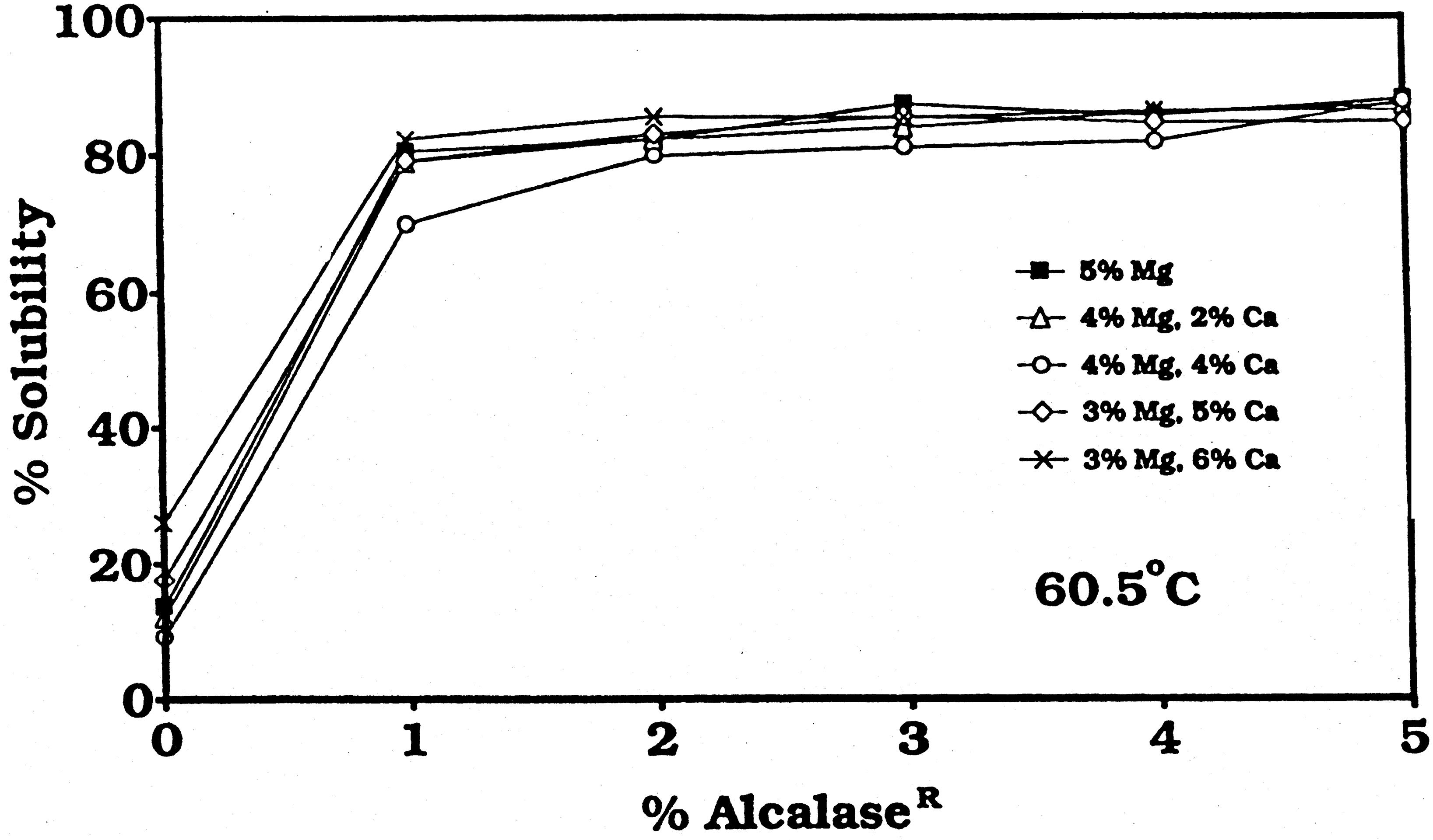


Figure 4. Effect of Alcalase® concentration, expressed as percent of wet weight, in the presence of varying magnesium oxide and lime combinations on the solubility of chrome shavings.

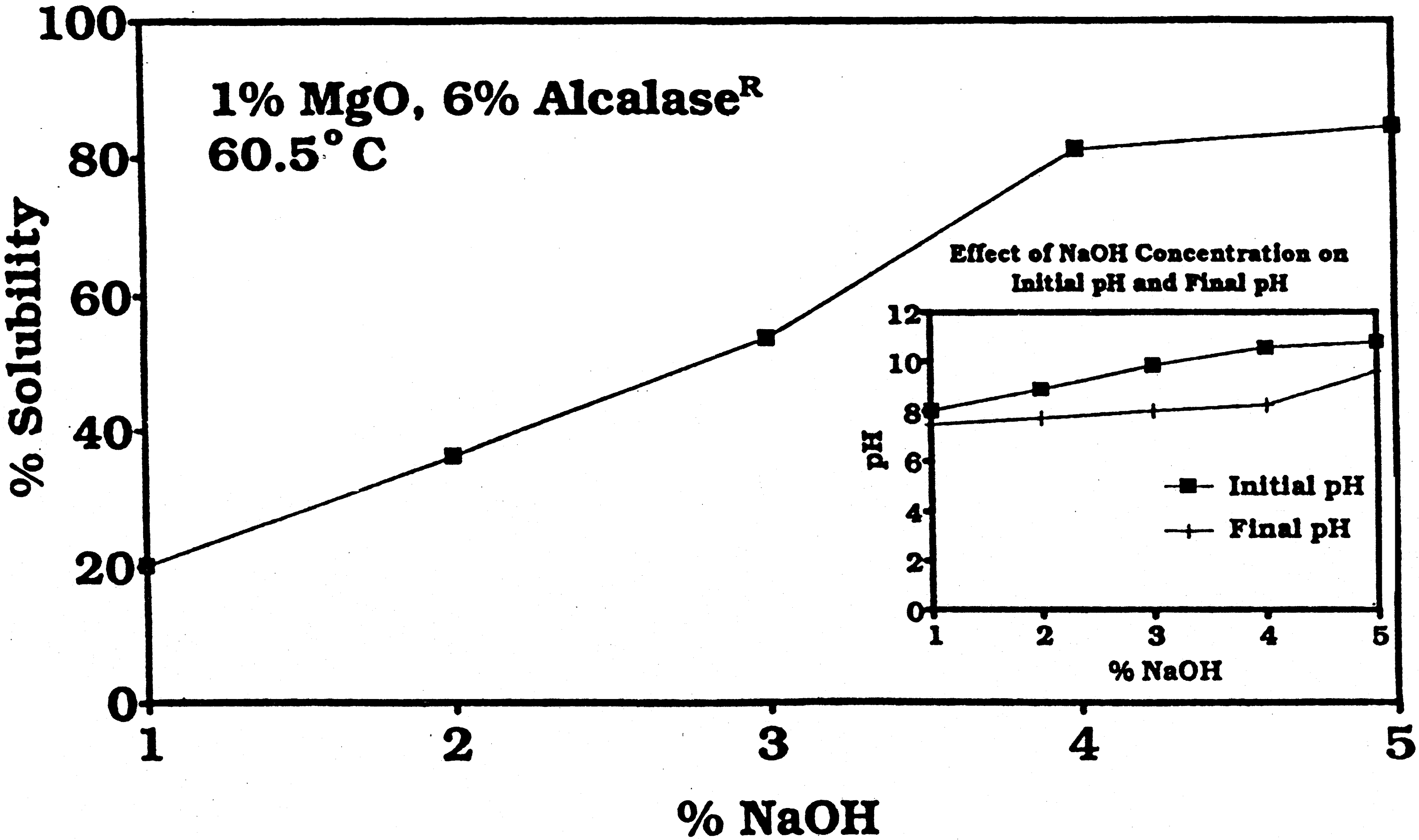
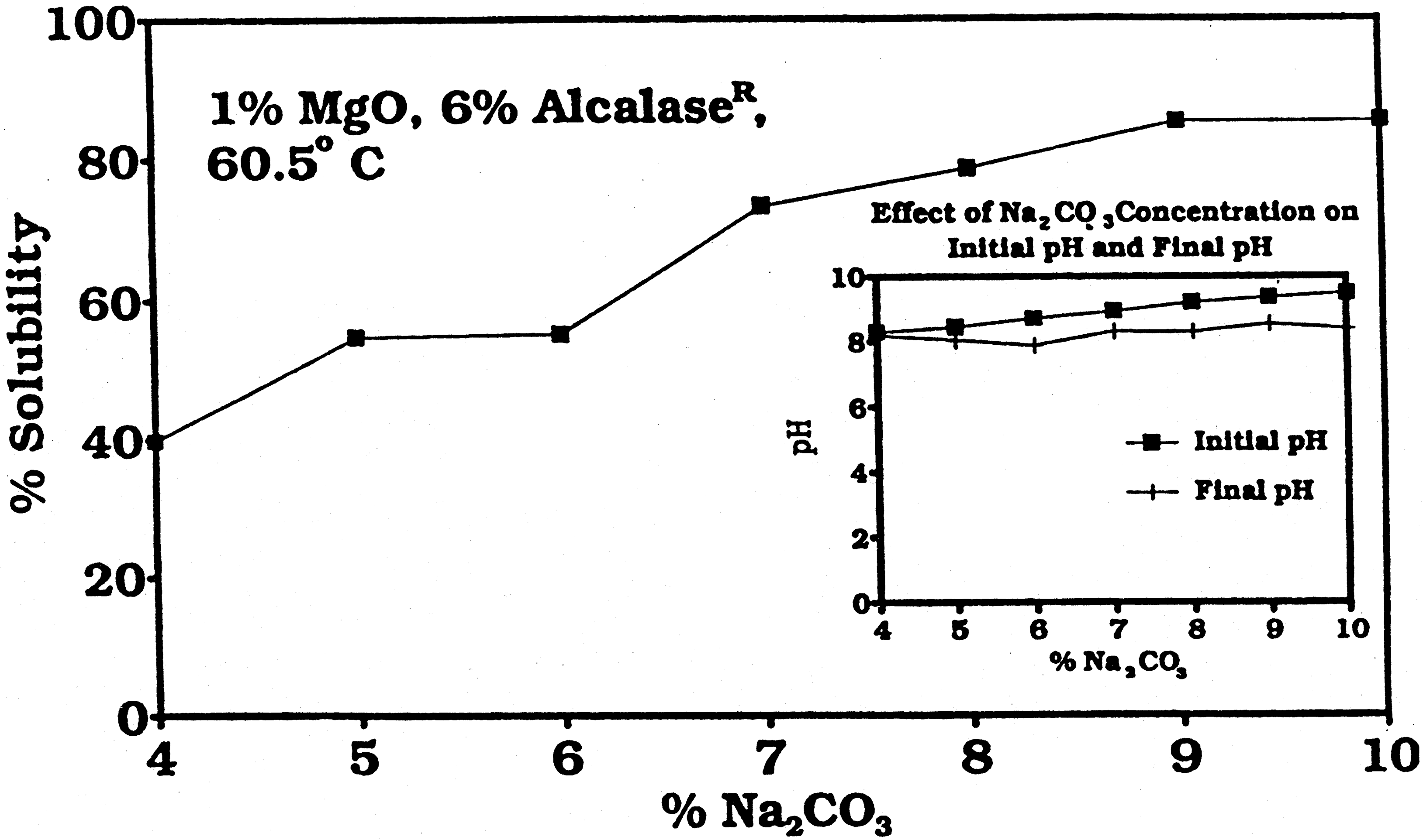


Figure 5. Effect of sodium hydroxide concentration, in the presence of 1% magnesium oxide and expressed as percent of wet weight, on the solubility of chrome shavings and on the initial and final pH's of the treatment.



6. Effect of sodium carbonate concentration, in the presence of 1% magnesium oxide and expressed as percent of wet weight, on the solubility of chrome shavings and on the initial and final pH's of the treatment.

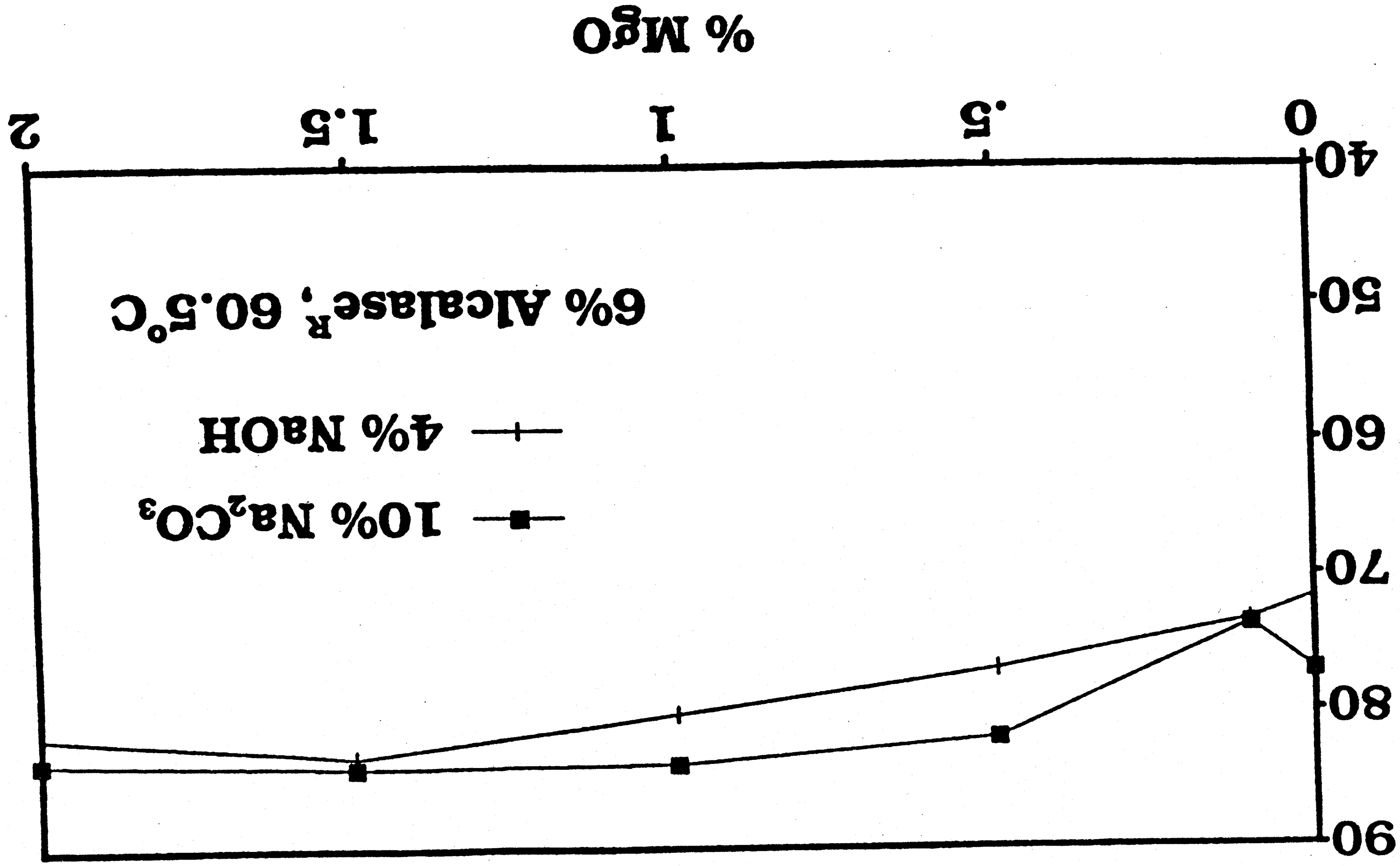


Figure 7. Effect of magnesium oxide concentration with 4% sodium hydroxide and 10% sodium carbonate, all expressed as percent of wet weight, on the solubility of chrome shavings.

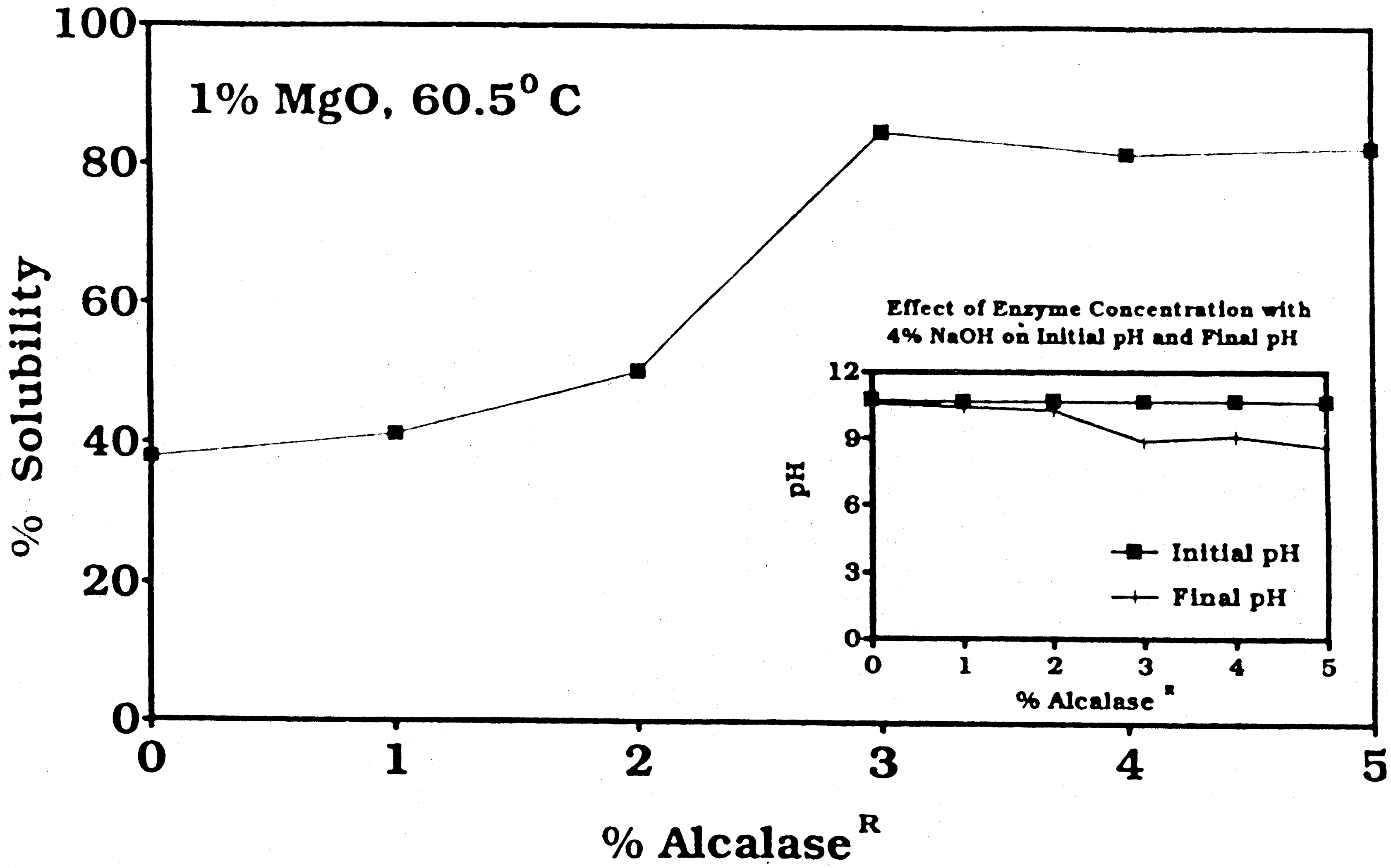


Figure 8. Effect of Alcalase® concentration with 4% sodium hydroxide, in the presence of 1% magnesium oxide and expressed as percent of wet weight, on the solubility of chrome shavings and on the initial and final pH's of the treatment.

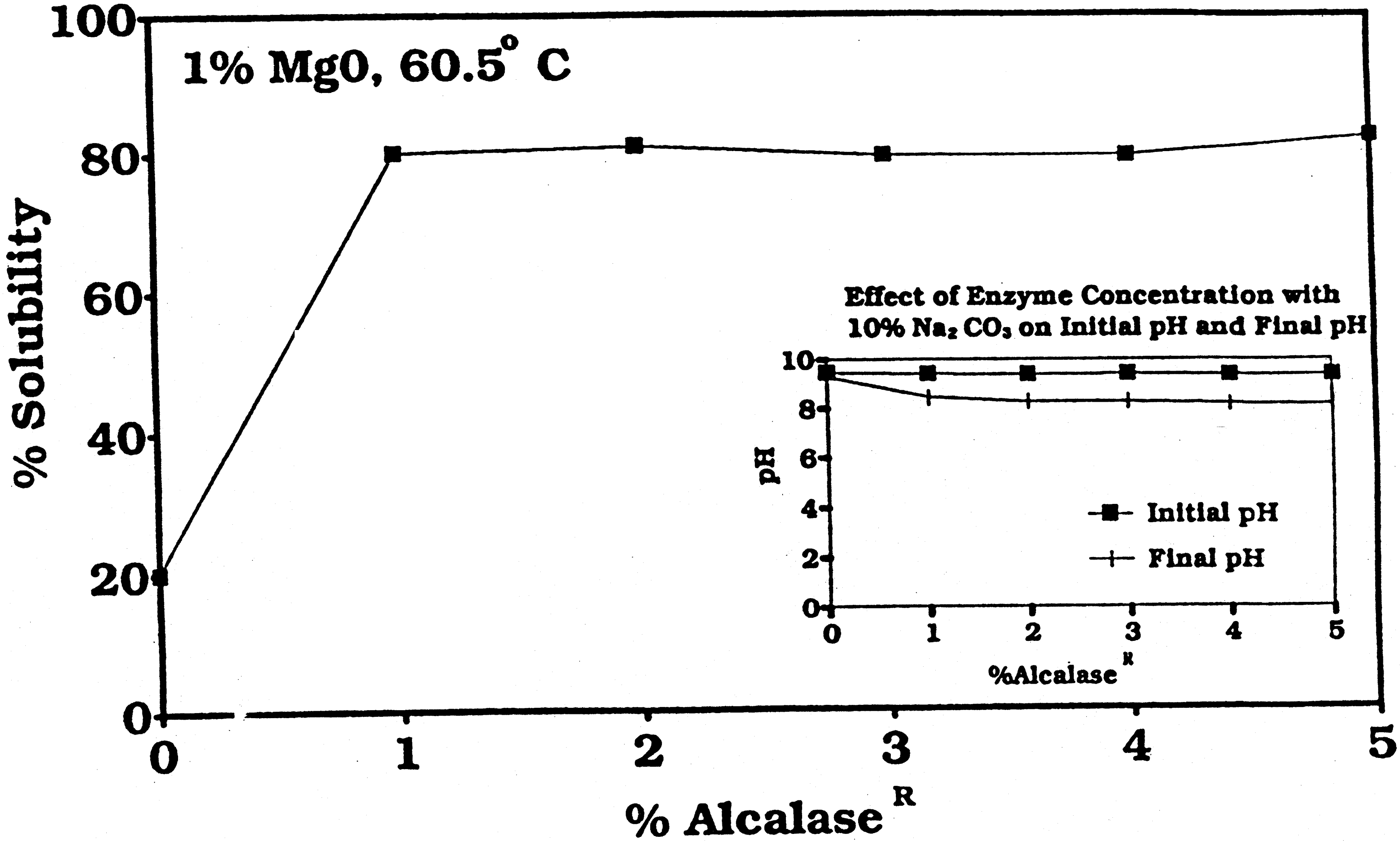


Figure 9. Effect of Alcalase® concentration with 10% sodium carbonate, in the presence of 1% magnesium oxide and expressed as percent of wet weight, on the solubility of chrome shavings and on the initial and final pH's of the treatment.