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Turbidity Reductions by Enzymatic Hydrolysis

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Turbidity Reductions By Enzymatic Hydrolysis

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ABSTRACT

Turbidity has long been a problem to the paper industry. In the past, it has been mainly associated with secondary fiber mills, among others. However, with the recent increase in the price of virgin pulp, other mills are seriously considering secondary fiber as a substitute for virgin pulp. Therefore, turbidity may become a problem to a larger number of mills in the future. Turbidity arises when starch and a filler are sheared together in a system. It has been noted in the literature that hypochlorite-oxidized starch produces the greatest turbidity phenomenon.

This project assumed that the size of the starch polymer was largely responsible for this effect and attempted to reduce the chain length through enzymatic hydrolysis. Initial hypochlorite-oxidized starch-titanium dioxide turbidities of 2200 JTU were reduced to 40-60 JTU through the use of alpha and beta amylases.

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INTRODUCTION

The presence of hypochlorite-oxidized starch in the white water systems of papermills, has been demonstrated to reduce filler retention and increase white water turbidity. Turbidity is an expression of an optical property of the fine suspended matter in a sample.

The papermaker is confronted with turbidity when a shearing force is exerted on the starch in the presence of a filler, particularly titanium dioxide. This would occur at the wet end when refining the stock and adding a starch and a filler at the same time. Starches have many applications in the paper industry today. They are usually used in the four following areas: (1) At the wet end; (2) At the size press; (3) At the calender stack and; (4) In the coating operation.

Secondary fiber mills seem to have the greatest problem with turbidity even though they may not use starch in their own system. This is because when reprocessing the secondary fiber, the starch may enter the system and upon shearing the the starch and filler together the turbidity arises. Fine paper mills also have a turbidity problem arising from their coating operation.

Sedimentation problems are also associated with turbidity because the fine matter is kept in suspension by the starch. This fine matter can be difficult to remove in the primary clarifier and can go on to the secondary treatment unit. This exerts an extra suspended solids load on the secondary treatment unit. Reduction of this suspension is also important for better setting rates and increased efficiency of the primary clarifier.

Turbidity also has an effect upon the receiving stream. It is unacceptable to the public because of its effect on the aesthetic value of the receiving stream. Turbidity can also cause a decrease in the photosynthetic process of the plant life in the stream. This could eventually upset nature's balance in the stream and begin to affect the animal life as well.

In the past, many methods have been tried to remove this turbidity from the suspension, but have met with little success. Some of these methods were polymers, radiation and ultrasonic degradation. Their drawbacks were cost, long degradation time. This project used enzymes to reduce the turbidity in a system by enzymatic hydrolysis to breakdown the starch polymer. This should cause it to become soluble. There are several reasons for using enzymes: (1) Enzymes do a similar function in the human body: (2) Enzymes are a vital part of secondary treatment systems for oxidation of organic matter to carbon dioxide and water and: (3) Enzymes are presently being used to convert pearl starches to enzyme converted starches.

OBJECT AND SCOPE

The purpose of this research was to reduce the effect of starch in peptizing starch-filler systems by enzymatic hydrolysis of the starch polymer. This would then allow sedimentation of the titanium dioxide filler particles, thereby reducing or eliminating turbidity of papermill effluents.

This system included simultaneous shearing of titanium dioxide and hypochlorite-oxidized starch. Also included was an evaluation of four enzymes, alpha and beta amylases to determine which enzyme was the most acceptable to the conditions of this project.

HISTORICAL BACKGROUND

Practical and laboratory experience has shown that hypochlorite-oxidized starch, even when present in small amounts, causes a reduction in filler retention, thus increasing white water turbidity. Brill stated previously, that oxidized starches had the greatest effect on reducing titanium dioxide retention, even though oxidized and enzyme converted starch reduce titanium dioxide retention. He further stated that oxidized starch concentrations as low as .1% affected filler retention (4).

Herrick found that oxidized starch had the greatest dispersion power of the many converted starches he studied. The procedure he used was developed by Davis (5) and uses the Canadian Standard Freeness Tester to determine single pass filler retention. Herrick also studied the dispersion effect of the starches with many different fillers. In all cases oxidized starch showed a greater filler loss than any of the other converted starches tested (6).

Starch acts as a stabilizer in the colloidal system by forming a protective colloid around small particles. Since the charge on both the starch and the fiber are negative, they repell each other and prevent the attractive van der Waals forces from destabilizing the system. Coehn stated that the carboxyl groups on the starch molecule are responsible for the net negative charge of the starch suspension (7). This was done by measuring the turbidity caused by various starches with and without carboxyl and aldehyde groups present. He also states that the dispersing power of the starch is dependent on molecular size (1). Harmon (8) says that the dispersion power of oxidized

starches is caused by two factors: carbonyl groups and molecule size with the latter being the primary factor in the dispersion power.

Turbidity develops most noticeably when oxidized starch and titanium dioxide are sheared in the presence of one another. This explains why secondary fiber mills have a large turbidity problem. When the secondary fiber is processed in an attempt to free the fiber from the ink, filler and adhesive associated with it, the starch and filler are sheared together resulting in a dispersed starch filler complex. The type of mechanical equipment used in the process of waste paper that give a shearing effect are the hydrapulper or even the fan pump. Wilhelm found that by increasing the time of shear, the turbidity also increased. This held true even after one hour settling time with a coagulant. He compared this to shearing one white water component and adding the other unsheared component to it. He found that shearing of the filler and starch separately did not result in a peptized system. Only when sheared in the presence of one another did the phenomina of turbidity exist. Once the turbidity results, it is very difficult to remove. It has been stated that this is due to the ability of the starch molecule to form a protective colloid around the titanium dioxide particles and thus prevents flocculation of the titanium dioxide particles. When comparing different starches, it was found that turbidity occurred only when oxidized starch and titanium dioxide were sheared together (1).

In the past many attempts have been made to treat the

paper industry's problem of turbidity, but with little success. Some of these attempts include: Long chain organic polymers were used as retention aids to increase filler retention thus reducing turbidity. Two factors contributed to their failure, one being their cost and secondly, in the presence of hypochlorite-oxidized starch, the effectiveness was sharply reduced. Ultrasonic degradation of the starch polymer is accomplished by applying ultrasonic energy to the suspension. However, this only broke the starch molecule down to dextrins, which are still fairly complex polysaccharides. Also, only single oxygen bridges were broken by this treatment (1). Biological treatment has also been used to remove turbidity from white water systems. This mechanism includes adsorption of the starch molecule onto the sludge floc, enzymatic degradation of the starch and absorbtion through the cellular wall. This report indicated that this approach worked well with most starches except of the oxidized type. A detention time of four hours was sufficient in most instances for turbidity removal except in the case of oxidized starches. With uses of oxidized starches, a minimum detention time of twenty-four hours was necessary for effective turbidity reduction.(3).

STARCH

Starch is a carbohydrate, snythesized within the plant by combination or polymerization of dextrose. Starch has been shown to be a high polymer, built up by the chemical interlinking of simple dextrose units. The plant employs two different mechanisms to synthesize its starch. It may form long linear carbohydrate chains by the successive at-

tachment of several hundred dextrose units. This is called the amylose or linear fraction. Another mechanism is to build a linear chain of maybe a dozen dextrose units, then a second mechanism may intrude to attach a dextrose unit in an off-shooting position. This is called the amylo-pectin or branched fraction. Most of the common starches contain both amylose and amylopectin fractions. The proportion of these fractions is different for each species of starch. Corn starch contains about 27% amylose, potato contains about 22% amylose and tapioca contains about 17% amylose (10). The structure of the amylose fraction of the starch can be seen in figure 1. It is connected by α-D- 1-4- linkages of dextrose units. The structure of the amylopectin fraction is shown in figure 2. It is connected by both α-D-1-4 linkages and α-D-1-6 linkages of dextrose units. Figures 3 and 4 show amylose and amylopectin, respectively, in their more familiar Fisher Formulas. Hanes suggested that the amylose molecule is coiled in the form of a helix with six glucose units per turn of the helix (11).

Starch is used in many industries today, basically because it is one of the most abundant materials in nature's storehouse. It is classed as a renewable substance since it is obtained from plants. Thus unlike minerals which are deposited in the earth's crust over long periods of time, a new supply of starch is grown annually. The four types of starches most commonly used in the paper industry are corn, potato, tapioca and wheat, in that order. Their differences lies in the physical and chemical characteristics of their individual starch granules. Table 1 gives

Figure 1. Linear Amylose Helix

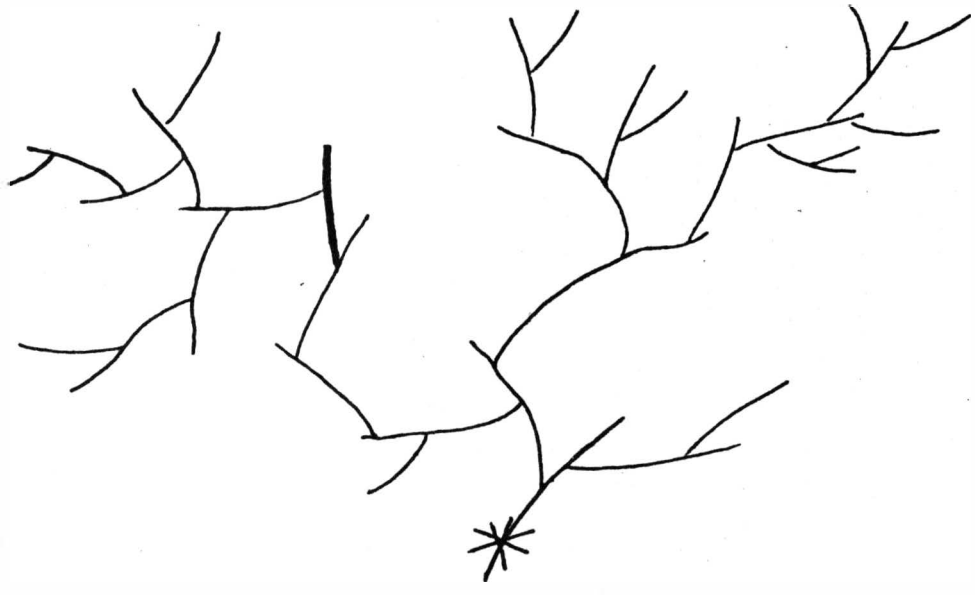

Figure 2. Branched Amylopectin

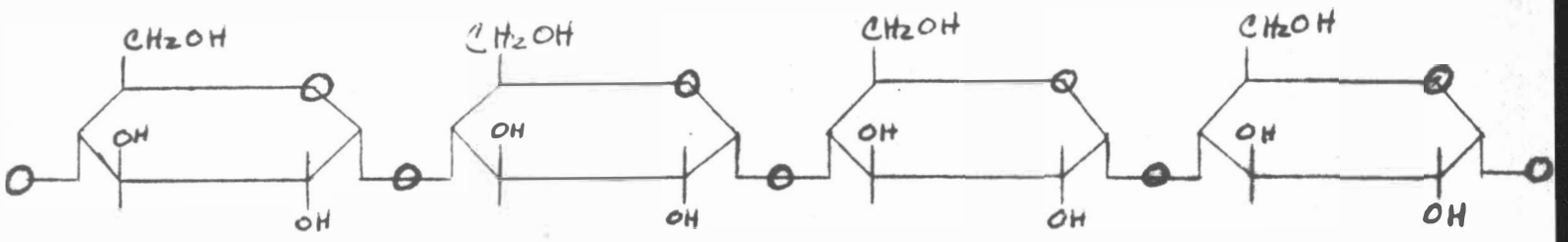

Figure 3. Amylose

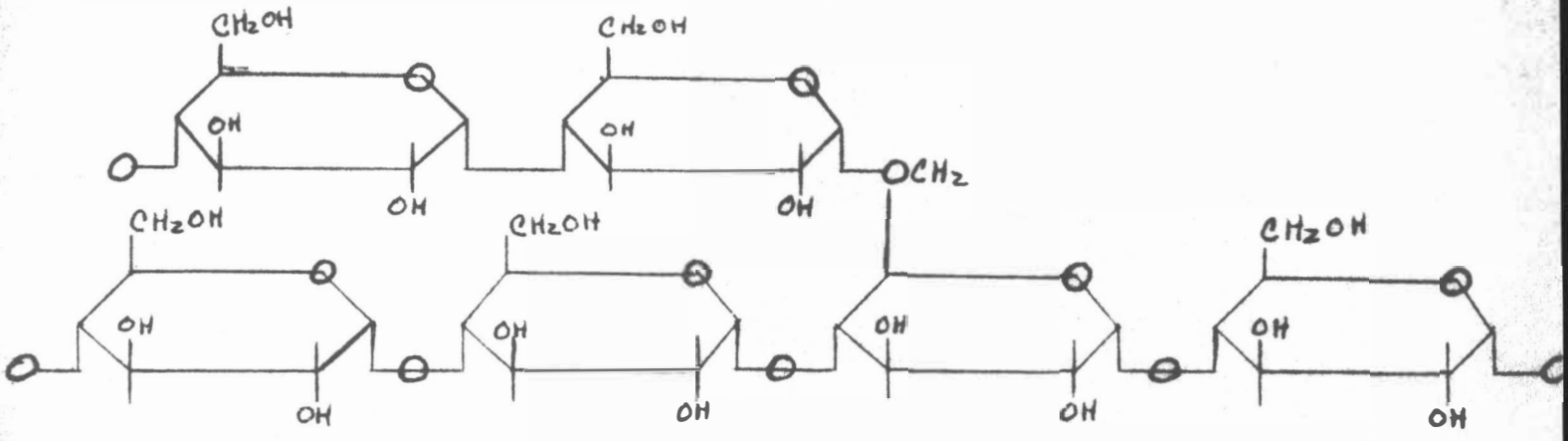

Figure 4. Amylopectin

the consumption of each starch in the paper industry in 1960, 1965, and 1968. In the last few years, however, corn starch has been mainly the only kind of starch used in the industry. This is due to the fact that corn starch is the most readily available of all the starches.

There are many types of modified starches used in the paper industry, among them are: hypochlorite-oxidized starches, pearl starches, acid converted starches, and hydroxyethylated starches. Their prices of these starches in 1969 are listed in Table 2. Hypochlorite-oxidized starches are used the most because they are the only starch commercially available on any large scale. Although other starch modifications, like cationic starch, are coming into wider use because of the turbidity problem associated with hypochlorite-oxidized starches. Hypochlorinated oxidized starches are made by adding sodium hypochlorite to a suspension of starch in water and heating it, to a temperature below the gelatinization point. The hypochlorite, by its oxidizing action, introduces zigzag discontinuities into the linear molecules so that they no longer will associate into micellar bundles. This modification increases the stability of the starch against gelling. In general, the pastes of oxidized starches are considerably more stable than those of thin-boiling starches. They are also clearer and more stable against gelling. The paper industry is a large consumer of the highly oxidized types for clay-coated paper because oxidized starches mix readily with clay and make an excellent binder (11).

The dispersing power of the oxidized starches is a

Table 1 (2)

Consumption of Starch in The Paper Industry (Tons)

Type	1960	1965	1968[a]
Corn	200,000	195,000	736,000
Potato	51,000	48,000	82,000[b]
Tapioca	46,000	95,000	
Wheat	24,000	6,000	
Coating & Converting	120,000	150,000	
	441,000	494,000	818,000

a: figures include coating and converting

b: figure combines potato and tapioca starches

Table 2 (10)

Cost of Some Starches

Type	Cost
Pearl Starch	$4.45/cwt
Acid Converted	$4.65 - $5.45/cwt
Oxidized Starch	$5.65 - $6.45/cwt
Hydroxyethylated Starches	$7.15 - $8.15/cwt

function of two variables: molecular weight and carbonyl groups. This causes the starch to keep it and other particles in suspension. Turbidity can occur anytime a starch and a filler titanium dioxide, are sheared in the presence of one another. This happens most often in secondary fiber mills. One way to combat this situation is to shorten the chain of the starch which is in effect reducing the molecular weight of the starch. This will decrease the dispersing power of the starch. This can be done with enzymatic hydrolysis of the starch chain. This will cut the chain length of the starch and hopefully reduce the turbidity. We attempted to do this so that it can be used economically at the paper mill level.

ENZYMES

Enzymes are the most efficient catalysts known. They speed up the rates of chemical reactions many times, and do so in a selective manner. Both the forward and reverse reactions are speeded up so that equilibrium is reached much faster than in non-catalized reactions, however the equilibrium remains the same. Catalysts do not appear in the end product and are not used up in the reaction. A catalyst causes the rate of a reaction to increase by reducing the energy of activation for that reaction. The specificity of enzymes toward substrates determines what organic or inorganic substance can be utilized by the cell. The first step in enzyme catalysis is the formation of an intermediate form or enzyme-substrate complex. The active center is where the reaction takes place on the enzyme. Here the substrate must have a perfect fit. This accounts for the limited range of substrate reacting with one enzyme. This

is similar to a lock and key relationship. In the final step of the reaction, the enzyme-substrate complex decomposes to yield product and free enzyme. No product can be formed except through this route.

Enzymes are important to humans because of their metabolic function in the body. Life is a combination of interlocking chemical reactions of the cell. All of these reactions are determined by the amount and nature of the enzymes present. The orderliness that characterizes chemical reactions in the cell is a consequence of enzyme specificity. Reaction specificity is that an enzyme can determine the product that should be formed from a given substance.

Enzymes are applicable to industries other than the Paper Industry. A few of the industries that use enzymes are: The Baked Goods Industry, Beer Industry, Dairy and Meat Products, Fruit Juice Industry and many others(12).

Temperature, pH, activators and inhibitors are all factors which influence the enzyme reaction velocity. One other factor which should be taken into account is the concentration of the enzyme. Temperature has a profound effect on the reaction velocity. In the temperature range of 20-30°C, there is little denaturation of the enzyme and the velocity of the reaction increases with increasing temperature. But at temperatures over 40°C, enzymes begin to denature, thus losing their activity. Therefore what is gained in velocity by increasing the temperature is lost by denaturation of the enzyme. All enzymes reach their maximal velocity at some pH range. Some enzymes denature and lose activity at pH's where others are quite stable. Many enzymes do not function optionally or not at all until a second substance has been added. These added substances are

called activators. Activation by inorganic ions is quite common and when an activator is needed, the rate of the enzyme reaction is dependent upon the concentration of the activator. Inhibitors stop or slow down an enzyme reaction. They are effective in small amounts. There are two types of inhibitors: Competitive and non-competitive. A competitive inhibitor has a structure similar to that of the substrate. The inhibitor combines with the active center of the enzyme to form an enzyme-inhibitor complex. Since the rate of the reaction depends on the concentration of the enzyme-substrate alone, one can calulate the extent of inhibition. Non-competitive inhibitors bind firmly to the enzyme and are not displaced by the addition of substrate. Generally, they are quite reactive, and attack functional groups of enzymes under very mild conditions. The greater their affinity for the enzyme, the greater the extent of inhibition(13).

HYDROLYTIC ENZYMES

Hydrolytic enzymes capable of catalizing the reduction of the starch polymer occur in nature, the digestive tract of animals, and within the cells of most plants and organisms. The four known types are: Alpha, beta, and gluco-amylases and oligo-saccharide hydrolysases. Only alpha and beta amylase were considered since they are more readily obtained from their natural environment.

Alpha amylase effects a rapid fragmentation of the starch polymer by hydrolysis of the α-D-(1-4) linkages randomly, while beta amylase sequentially reduces the non-reducing end of both the linear and branched portion of the starch polymer, as demonstrated in Figure 5. Both enzymes halt their action when they encounter the α-D-(1-6) linkage. The remaining fractions are called limit dextrins,

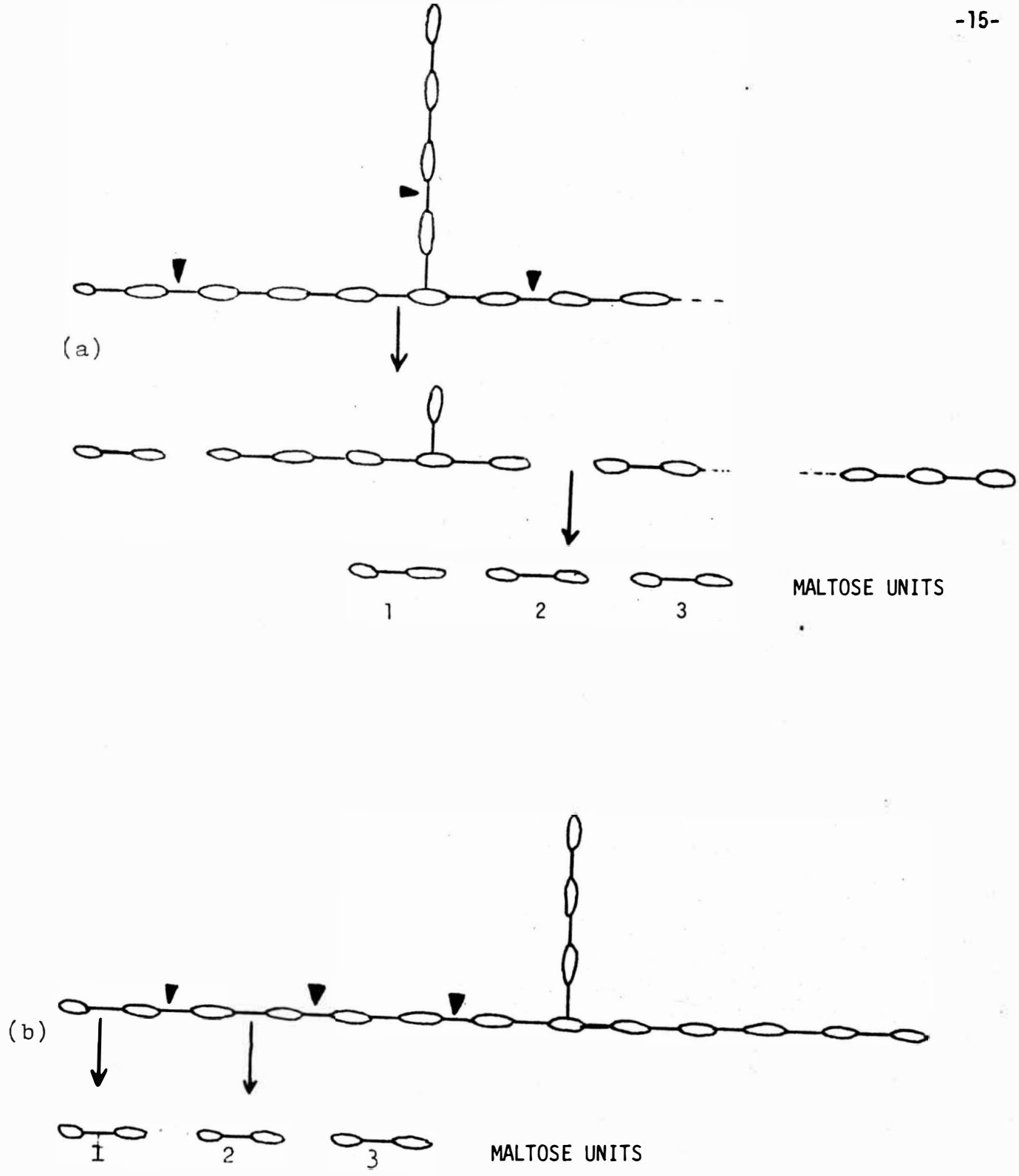

Figure 5. Modes of attack of (a) alpha amylase and (b) beta amylase

and also contain some ∝-D-(1-4) bonds. The initial action of the amylase is the rapid hydrolysis of starch into low molecular weight oligosaccharides(saccharides that contain a known small number of monosaccharide units) with the subsequent hydrolysis to D-glucose and maltose as shown in Figure 6.

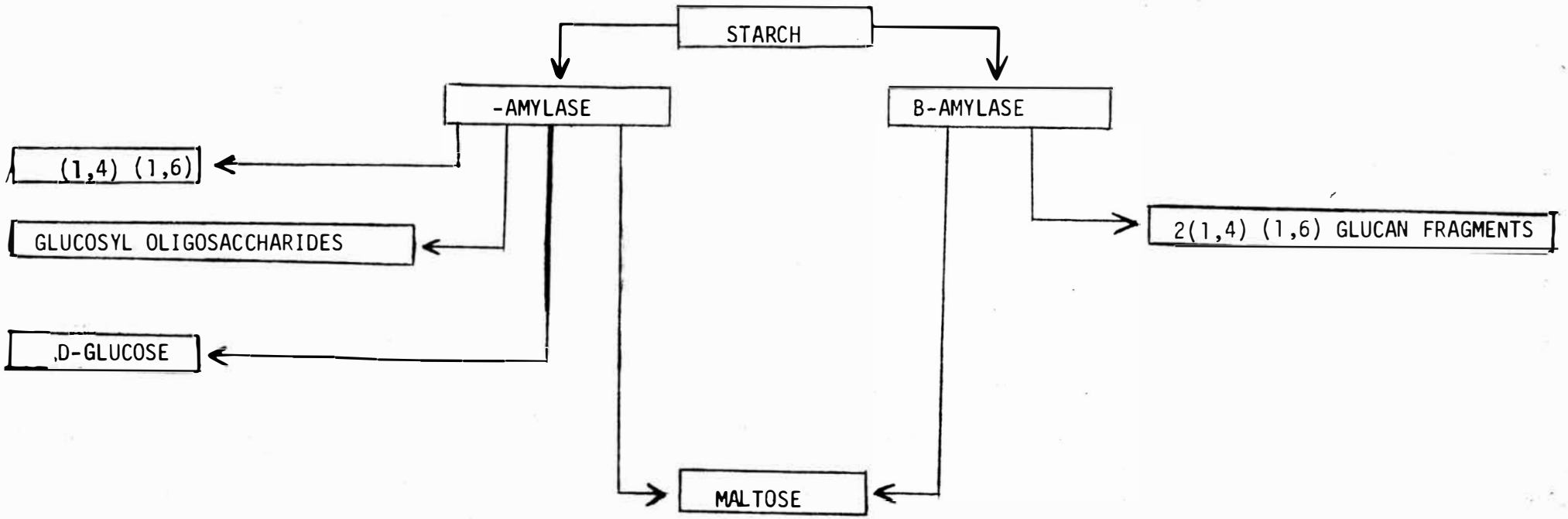

Figure 6. Amylase Action Upon Starch

LABORATORY PROCEDURE

Throughout these experimental runs, four variables were held constant: (1) Reaction temperature, 86°F, (2) Reaction time, two hours, (3) The pH levels were 6.6 and 7.0, and, (4) The substrate concentration. The reaction temperature of 86°F, was selected as being the average mill effluent temperature. A reaction time of two hours was selected since it represented approximately 50% of the minimum detention time of most clarifiers. The pH of the reaction vessels were adjusted to the recommended manufacture level for maximum efficiency. Commercial enzymes, amylases, presently available to the paper industry, were used in this project.

Hypochlorite-oxidized starch was cooked at 20% solids, for fifteen minutes at 200°F in a pan heated by a bunsen burner. The starch was then diluted to 2% solids by the addition of distilled water. 500 milligrams of titanium dioxide was added to 500 milliliters of the 2% starch, placed in an eight speed Waring Blender and sheared for ten minutes at the lowest speed. The suspension was then placed in a water bath to allow it to acclimate at 86°F.

The next step was to prepare the four enzyme solutions that were to be evaluated. This was done by diluting two grams of each enzyme concentrate to 50 milliliters in a volumetric flask with distilled water. The enzyme concentrates used were:

A) Alpha-amylase
B) Alpha-amylase
C) Alpha-amylase
D) Beta-amylase

The experimental run was begun by setting up nine test tubes in a rack; one rack for each enzyme solution. Ten milliliters of the prepared starch-titanium suspension were added to each test tube. To the test tubes, varying ratios of enzyme solution and distilled water, were added so that the total volume in each test tube was fifteen milliliters. The test tubes were allowed to react for the two hour reaction period. At the end of the reaction period, the test tubes were removed and examined visually. Two criteria were used in selecting the most efficient enzyme: (1) What enzymes did the best job of reducing the turbidity with in the two hour period and, (2) The maximum efficiency of turbidity reduction at minimum enzyme concentration.

On this basis, enzymes A and D were selected and the experimental conditions were scaled up to 500 milliliters of hypochlorite-oxidized starch and ten grams of enzyme concentrate per reaction beaker. Turbidity and viscosity measurements were made before and after the enzyme hydrolysis with the Hach Turbidimeter and Brookfield Viscometer respectivity. The enzyme to starch ratio was kept at the minimum enzyme concentration for maximum efficiency as was previously determined in the test tube evaluation. Three 600 milliliter reaction beakers were used in this evaluation. Two were for the enzyme additions and the third beaker was used as a control in the evaluation. The reaction beakers were acclimated to 86^{o} and the selected enzymes were added and allowed to react for two hours.

A list of materials and equipment used throughout this project are listed in Appendix A.

DISCUSSION

Figures 7 and 8 show the results of the alpha amylases, (A) and (B), after the two hour reaction period. Figures 9 and 10, show the alpha amylase (C) and the beta amylase (D) after a three and one half hour reaction period. (A picture at a reaction period of two hours was not obtained.) These figures show that the alpha amylase (A) and the beta amylase (D), did the best job of reducing turbidity of the four enzymes evaluated. The maximum turbidity reduction at minimum enzyme concentration for these two enzymes was .2 grams of enzyme concentrate to ten milliliters of the starch-titanium suspension.

An interesting observation of the method in which the enzyme attacked the suspension was first noticed in the test tube evaluation and again in the scaled up evaluation. The enzyme seemed to attack in three stages: (1) Coagulation of the suspension: (2) settling out of the coagulated material and: (3) the final degradation of the settled material.

Figures 11 and 12, show enzymes A and D after a reaction period of 24 hours. It can be seen from these figures that after 24 hours of reaction time, the turbidity of the suspension was completely reduced at all levels of enzyme concentration.

When enzymes, A and D, were used on the larger volumes and turbidities and viscosities measured, the results were:

ENZYME	TURBIDITY (JTU)		VISCOSITY (CPS)	
	Before	After	Before	After
A	2200	80	20	16
B	2200	40	20	12

TABLE III

Figure 7. Alpha amylase (A) after two hour reaction period.

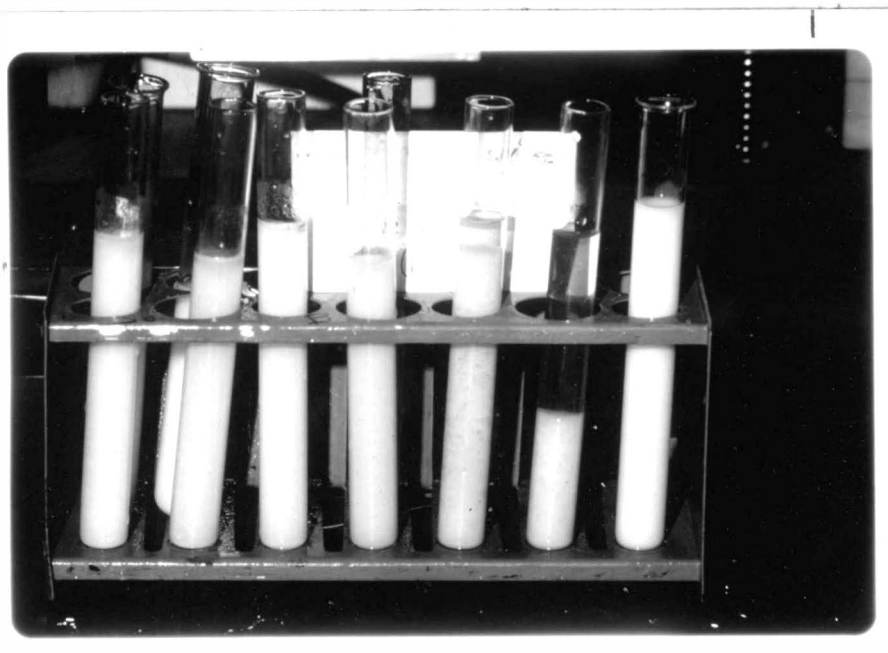

Figure 8. Alpha amylase (B) after two hour reaction period.

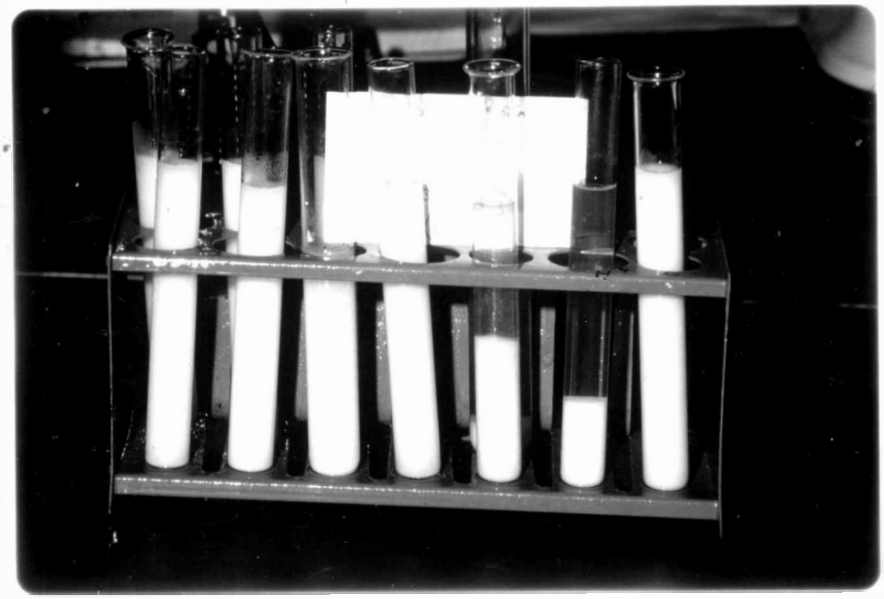

Figure 9. Alpha amylase (C) after 3½ hour reaction period.

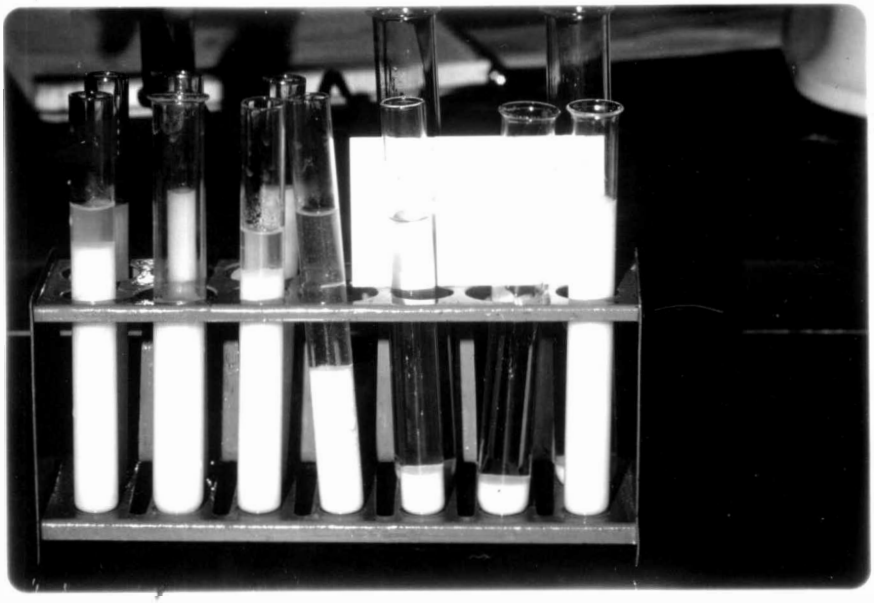

Figure 10. Beta amylase (D) after 3½ hour reaction period.

Figure 11. Alpha amylase (A) after 24 hours of reaction.

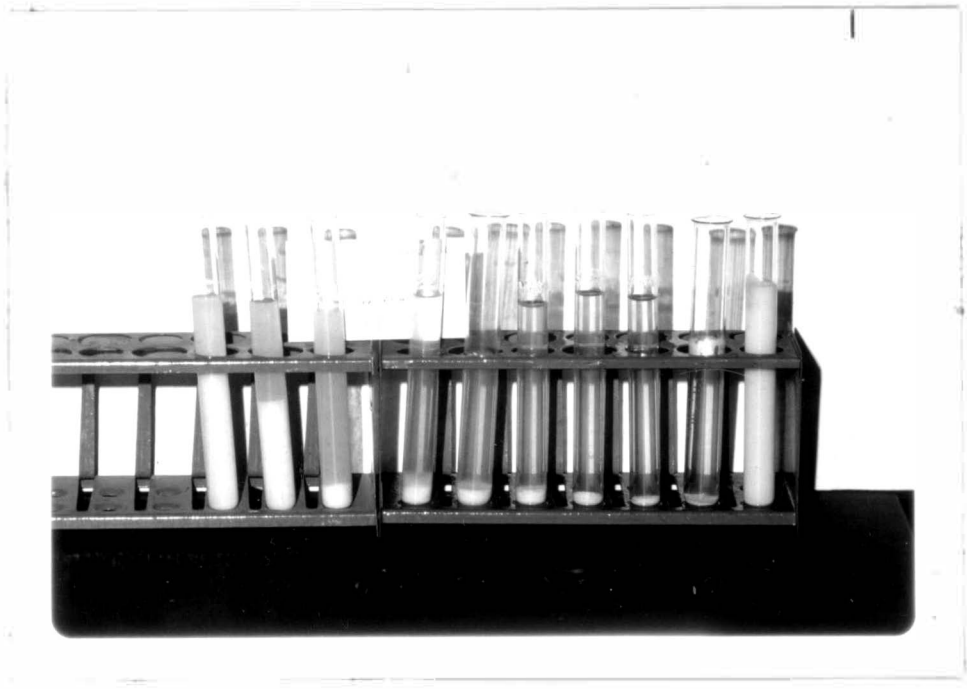

Figure 12. Beta amylase (D) after 24 hours of reaction.

Table III showed that the beta amylase,(D), reduced the turbidity to a lower level than did the alpha amylase,(A). Using viscisity as an indication of chain length, again the beta amylase did the best job in shortening the chain length. From these results, it appeared that the beta amylase did a better overall job of removing turbidity than did the alpha amylase. Figures 13, 14, 15, 16 and 17 show the scaled up reaction at .67, 1.00, 1.50, 2.00 and 24.00 hours, respectively.

One final evaluation of the enzymes was made and that was of their cost. The following Table lists their prices:

ENZYME	COST	
	5 GALLONS(¢/lb.)	55 GALLONS(¢/lb.)
A	90	80
B	90	80
C	90	80
D	73	64

TABLE IV

It can be seen from Table IV that, not only is the beta amylase the most efficient enzyme in reducing turbidity but, it is also the most economical.

An attempt was made to follow the degradation of the starch polymer through the use of the starch-iodine-iodate color complex. However, it was found that the starch in the supernatant was broken down beyond the polymeric length and would not react with the iodine solution to give a color range.

One negative result in this project was that a brown color was left in the supernatant after the reaction was finished. This can be seen in all the pictures throughout the experiment. It was felt to be caused by impurities in the enzyme solution itself and that by increasing the reaction

Figure 13. Enzymes, (A) and (D), after .67 hours reaction.

Figure 14. Enzymes, (A) and (D), after 1 hour reaction.

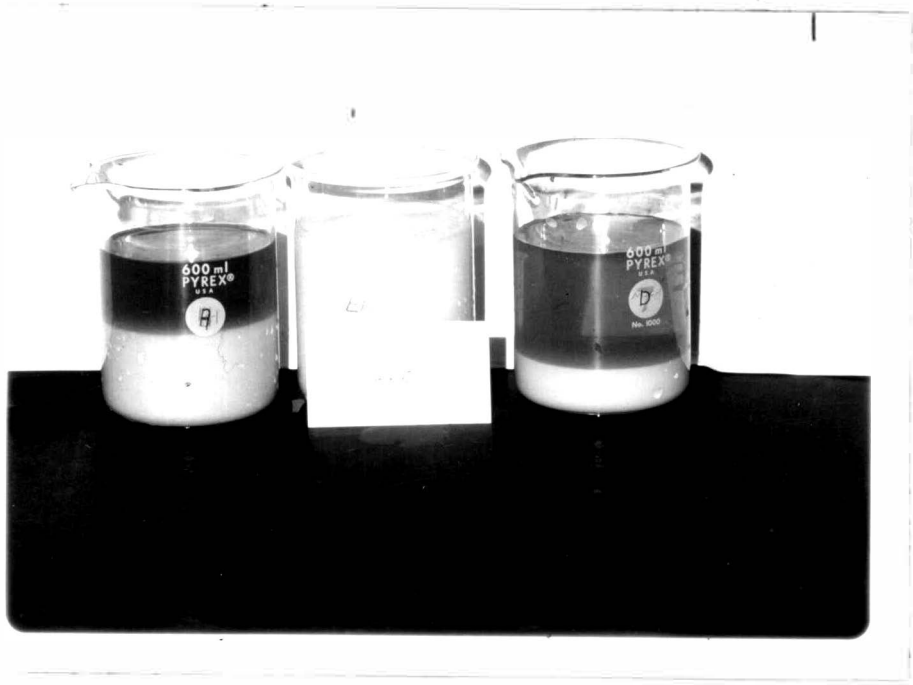

Figure 15. Enzymes, (A) and (D), after 1.5 hours reaction.

Figure 16. Enzymes, (A) and (D), after 2 hours reaction.

Figure 17. Enzymes, (A) and (D), after 24 hours reaction.

temperature, this brown color could possibly be eliminated.

The results of this project could have some of the following possibilities if applied at the paper mill level. In a hypothetical mill that produces 100 tons/day and uses 1000 gallons of water/ton, the cost would be approximately $11,000/day. (Assuming the mill effluent had the same conditions as this project). However, by treating the effluent with this enzyme, it could allow this mill to reuse it's effluent back into the system. The cost of using this enzyme might be reduced because the enzyme could build up in the white water system and the daily dosage level would become smaller. By having this enzyme in the system, the filler retention might increase. If the white water isn't being reused, then addition of the enzyme to the effluent could increase the efficiency of the primary clarifier. Since the enzyme seemed to cause the starch to coagulate, then it might increase settling rates of the clarifier by improving coagulation capabilities. Enzyme addition to the effluent might also decrease detention time in the secondary treatment unit because the starch coming to the unit would have already been partially degraded by enzymatic hydrolysis. Therefore lessening the job on the secondary treatment unit.

The high cost of the enzyme used in the hypothetical mill situation above bears further discussion. Carpenter and Janis stated that the starch concentration of a mill effluent rarely exceeded 800 ppm (3). The starch concentration of the above mill effluent was 20,000 ppm, which helps explain the high cost. Another method to reduce the enzyme cost would be to increase the temperature of the enzyme reaction, since theoretically increasing the temperature of a chemical reaction increases the rate of that reaction. This would reduce the amount of enzyme needed. One final possibility of reducing the enzyme cost would be to use the enzyme bonded to an insoluble carrier.

Smiley stated that the enzyme is more stable when bound to a carrier than when used in solution. The immobilized enzyme can be recovered with no loss of enzyme, and thus no additional cost to replace lost enzyme (14).

CONCLUSIONS

1. The turbidity of a hypochlorite-oxidized starch-titanium dioxide suspension was reduced by the use of alpha and beta amylases. During this turbidity reduction, the enzymes seemed to attack the suspension in three stages: (1) Coagulation, (2) settling out of the coagulated material and, (3) final degradation of the settled material.
2. In this project, the beta amylase was the most economical and most efficient in reducing turbidity over the three different alpha amylases evaluated.
3. Turbidity reductions can be achieved at almost any level of enzyme concentration dependent upon reaction time, temperature and the amount of substrate present.
4. The effect of using this enzyme on a paper mill's effluent under the same conditions as this project, might be in three areas: (1) Allowing the mill to reuse it's effluent in it's system and increase filler retention: (2) increasing the efficiency of the primary clarifier by increasing the settling rate of the clarifier and: (3) decreasing the detention time of the secondary treatment unit.

RECOMMENDATIONS

1. Further studies should be made to determine what effect the enzymes would have on reducing turbidities caused by starches other than hypochlorite-oxidized starch. This study should also cover the effect on a mixture of starches.
2. Work should be done to determine the effect of pH, and temperature changes on the efficiency of the enzyme in reducing turbidity. Included in this study should be an attempt to eliminate the brown color left in the supernatant by the enzyme.

APPENDIX A

LIST OF MATERIALS AND LABORATORY EQUIPMENT

Materials:

Commercial enzymes

A) Alpha-amylase

B) Alpha-amylase

C) Alpha-amylase

D) Beta-amylase

Hypochlorite- oxidized starch - Stayco M; A.E. Staley Manufacturing Company, Decatur, Illinois

Titanium Dioxide - Ti-Pure LW; E.I. Du Pont, De NeMours Company, Wilmington, Delaware

Equipment:

Eight-speed Waring Blender

Hach Turbidimeter

Brookfield Viscometer

Water Baths

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