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PREPARATION OF SULFATED CARBOHYDRATE FOR TREATMENT OF HIGH PROTEIN WASTEWATER.

THE UNIVERSITY OF OKLAHOMA, PH.D., 1979

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

PREPARATION OF SULFATED CARBOHYDRATE FOR TREATMENT OF HIGH PROTEIN WASTEWATER

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fullfillment of the requirement for the

degree of

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BY

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Norman, Oklahoma

1979

PREPARATION OF SULFATED CARBOHYDRATE FOR

TREATMENT OF HIGH PROTEIN WASTEWATER

APPROVED BY

Leslie E. Stern

James M. Robertson

Geo. W. [illegible]

Leon J. Cieyko

DISSERTATION COMMITTEE

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PREPARATION OF SULFATED CARBOHYDRATE FOR TREATMENT OF HIGH PROTEIN WASTEWATER

CHAPTER I

INTRODUCTION

The Federal Water Pollution Control Act Amendments of 1972, now officially designated Public Law 92-500, established a new wastewater discharge permit system to be administered by the Environmental Protection Agency (U.S. Congress, Senate, an Act To Amend the Federal Water Pollution Control Act, S.2770, PL 92-500, 92nd Congress, October 18, 1972). This law established a national goal that the discharge of pollutants into navigable waters be eliminated by 1985; that wherever attainable, an interim goal of water quality which provides for the propagation of fish, shellfish, and wildlife, and for recreation in and on the water be achieved by July 1, 1983; and that the discharge of pollutants in toxic amounts be prohibited.

According to PL 92-500, beginning July 1, 1977 the food process-industry was to apply the best practicable control technology currently available if their wastes were discharged into a watercourse. By July 1, 1983, the food processing industry must apply the best available technology (BAT) which will result in future progress toward the national goal of eliminating the discharge of all pollutants. In August, 1978,

Congress amended PL 92-500 in order to adjust water discharge regulations to meet realistic conditions of time and economics. Under the Clean Water Act of 1977 a new technology-based effluent limitation, best conventional pollutant control technology (BCT), replaces the general, more stringent, BAT currently covering conventional pollutants. BCT controls are due to be installed by July, 1984 for those discharges that are not involved in toxic substances, such as those from the food processing industry.

Recent surveys of the pollution load in meat-packing wastewaters show the importance of controlling this type of waste and the consequences it has on our wastewater treatment programs. In 1967 the potential daily Biochemical Oxygen Demand (BOD) from the slaughterhouse and meat-packing industry was estimated at 2.17 million pounds or a population equivalent of 13 million people (Dirasian, 1970). The U.S. Department of Agriculture places the meat-packing industry second only to the pulp and paper industry in terms of the potential BOD pollution.

The amount of solids, grease and nitrogen in wastewater has a significant effect on the treatment costs of sewage disposal systems. The solids contribute a heavy load to primary clarifiers and add to sludge disposal costs. Heavy loads of grease interfere with biological processes. Grease coats the media of trickling filters, thus reducing treatment efficiency. Removal of nitrogen from the wastewater is a very expensive process.

In recent years, methods have been developed for removal of protein from wastewaters resulting from the processing of animal, plant

and microbial materials. These methods include precipitation by sugar sulfate esters which consistently removes 70 to 80 percent of the BOD_5, and a high percentage of nitrogen, grease, suspended solids, and microorganisms. Following precipitation these materials can be separated by sedimentation, dissolved air flotation or centrifugation. The effluent is compatible with domestic waste and can be discharged directly to a municipal collection and waste treatment system. If a municipal system is not available, the waste can be biologically treated much more economically than raw waste, and subsequently discharged to receiving streams. In addition, the sludge obtained from protein precipitation can be recovered as a valuable by-product, which can be incorporated in animal feed.

The process is not presently being used in the United States due to the unavailability of the precipitating agent. D-glucose has been used as the starting material to prepare the sulfate ester in the laboratory for experimental purposes. Glucose, although readily available, is expensive and, therefore, not suitable for use as a starting material in large-scale production.

Sulfated carbohydrates may be prepared by several methods. The protein precipitation efficiency varies with the method employed. All methods result in a dilute solution, which is not practical for storage and transportation. It is the objective of this study to develop a method using inexpensive raw materials to prepare a sulfated carbohydrate and to transform the final product into a crystalline form.

CHAPTER II

LITERATURE SURVEY

The technology for treating food processing wastewater is available. Carbohydrate sulfate has been used as a precipitating agent to remove protein from food processing wastewater. To understand the principal of protein precipitation a knowledge of the general properties of proteins is essential.

Physical and Chemical Properties of Proteins

Proteins can behave as both acids and bases. Since proteins are electrolytes, they migrate in an electric field and the direction of migration will be determined by the net charge of the molecule. The net charge is influenced by pH, and for each protein there is a pH value at which it is not affected by an electric field; this pH value is the isoelectric point (IP). At pH values acid to the isoelectric point, the protein will have a net positive charge. Correspondingly, at a pH value alkaline to the isoelectric point the protein will possess a negative charge. The isoelectric point of a given protein is a constant (Sawyer, 1967).

The proteins are dipolar ions at the isoelectric point; i.e., the sum of the positive charges is equal to the sum of the negative charges and the net charge is zero. The number of ionized groups on the

protein molecule, the sum of positive and negative charges together, may be at a maximum at the isoelectric point. The actual numbers of positive and negative charges can be measured by titration of the protein.

Protein can form salts of two types; i.e., protein anions can bind with cations, and protein cations with anions. Many ions form insoluble salts with proteins and serve as excellent precipitating agents for proteins. Acids, such as phosphotungstic, trichloroacetic, picric, sulfosalicylic, perchloric, etc., are used for deproteinizing solutions. Heavy metal ions are used for precipitating proteins on the alkaline side of their isoelectric points, the protein behaving as anions. Ions of mercury, copper, silver, zinc, barium, etc., are frequently employed for this purpose.

The solubility of proteins is markedly influenced by pH; solubility is at a minimum at the isoelectric point, and increases with growing acidity or alkalinity (White, 1968). The explanation is as follows: In the isoelectric condition, electrostatic repulsive forces between solute molecules are at a minimum and crystal lattice forces in the solid state will be at a maximum. When the molecule exists predominantly as either anions or cations, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign and will be more soluble than in the isoelectric state.

It is apparent that certain factors greatly influence protein solubility. The major influences on solubility are considered below.

The solubility of protein is markedly increased by neutral salts. The effect of neutral salts in increasing the solubility of protein is

called the "salting-in" effect. The explanation of the "salting-in" phenomenon is as follows: Solubility of any substance depends on the relative affinity of solute molecules for each other (crystal lattice formation) and for the solvent molecules. Any factor that decreases interaction of solute molecules will tend to increase solubility. The small ions of neutral salts will interact with the ionic groups of the protein molecules, diminishing protein molecule interactions and, therefore, increasing solubility.

Proteins are precipitated from an aqueous solution by high concentrations of neutral salts. This is the "salting-out" phenomenon. Di- and trivalent ions are more effective than univalent ions. Commonly used salts are ammonium sulfate, sodium sulfate, magnesium salts, and phosphates. The most effective region of salting-out is at the isoelectric point of the protein. The mechanism of salting-out is complex. Hufmeister (1891) suggested that this was due to "dehydration" of the protein by the added salt. According to Debye, the salt ions attract around themselves the polarizable water molecules, making less water available for the protein, since, at high salt concentrations the number of charged groups contributed by the salts is enormous compared with those of the proteins. Since solubility of proteins in water depends on clustering of water molecules around the hydrophilic ionic groups, removal of water molecules to other ions will decrease protein solubility.

Proteins are precipitated by the addition of water-miscible neutral organic solvents, such as ethanol and acetone. Protein solubilities in these solvents are markedly affected by neutral salts.

Temperature also affects the solubility of proteins. Within

a limited range, from 0° to about 40° C., most proteins will increase in solubility with higher temperature, but there are exceptions. Above 40° to 50° C., most proteins become increasingly unstable and begin to denature, ordinarily with a loss of solubility in the neutral pH zone (White, 1968).

Colloid Chemisty of Protein

When soluble protein dissolves in water, it can be considered as a colloidal system. Proteins are solvent-loving colloids which are called hydrophilic colloids. When the protein colloids disperse in water, hydrated ions are absorbed by the protein colloids; therefore a positive charge is gained on the colloids. The stability of the protein colloids depends upon the electrical charge they possess. The stability of the colloid is generally a function of the magnitude of the charge, commonly referred to as the zeta potential ξ. The zeta potential is defined by the equation (Sawyer, 1967):

$$\xi = \frac{4\pi\delta q}{D} \text{ - - - - - - - - - - - - (1)}$$

Where q is a the charge on the particle, δ is the thickness of the zone of influence of the charge on the particle, and D is the dielectric constant of the liquid.

Therefore, the hydrophilic colloids possess two factors of stability, an electric charge and a hydration shell. Many hydrophilic colloids may be brought to the isoelectric point and still remain relatively stable, stabilized by the hydration shell.

The destruction of the protein colloid may be accomplished in several different ways. Four methods are (1) boiling; (2) freezing;

(3) addition of electrolytes; and (4) mutual precipitation by addition of a colloid of opposite charge. The last method is the most promising in engineering practice and will be discussed further.

Mutual precipitation occurs when colloids of opposite charge are mixed. A special application of this phenomenon is called coacervation. The phenomenon of coacervation is the separation of microscopic liquid droplets when two hydrophilic colloids unite to form a viscous liquid layer at the bottom of the container. Gelatin and gum acacia colloids may be taken as representative systems which form coacervates (Gortner, 1949). Figure 1 shows diagrammatically the conditions which must be met. Gelatin, in common with most proteins, assumes either a positive or a negative charge, depending on the hydrogen-ion concentration of the system. At a pH greater than 4.7 gelatin is negatively charged. At a pH below 4.7 the colloids are positively charged. The reversal of sign of gelatin is shown by curve A, Fig. 1. Gum acacia retains its negative charge over a wide range of hydrogen-ion concentration, as indicated by curve B, Fig. 1. At the right of the line xy both gelatin and gum acacia are negatively charged. The two systems do not interact when mixed, and the relative viscosity of the mixture is the average of the relative viscosities of the colloids used. To the left of line xy, Fig. 1, mutual precipitation will occur between the gelatin and gum acacia colloids, but because of the water shell surrounding the individual colloids they cannot coalesce with complete destruction of the double layers, but are held apart by the resistance of the water shells. Electrostatic forces tend to cause aggregation,

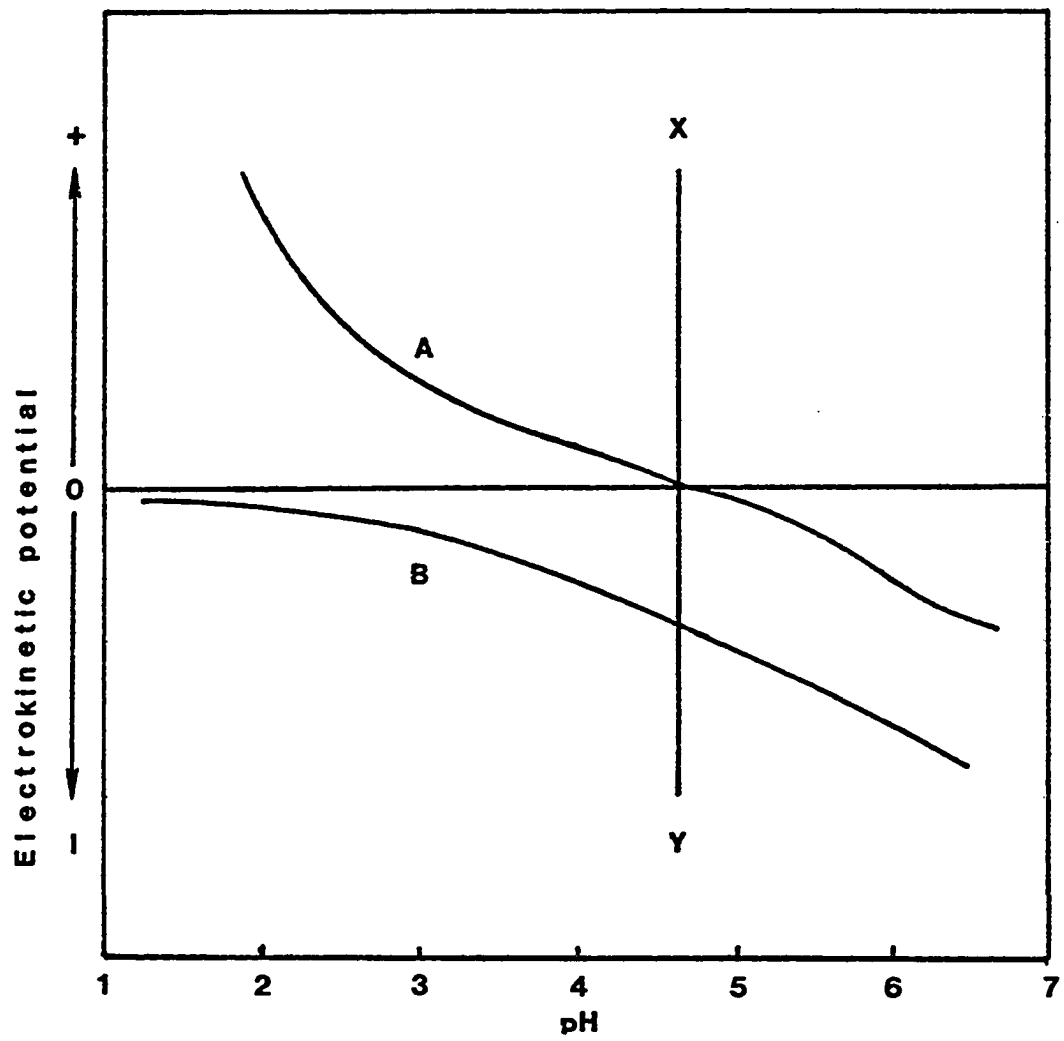


Figure 1. Effect of pH on the Additive Viscosity and Coacervate Formation.

but these forces are resisted by the elasticity of the water shells surrounding the hydrophilic colloids, so that the individually charged colloids retain their identity, but are held together in a swarm by electrostatic attractions. The net result is that the droplets of liquid separate as a new phase.

Since the force of attraction which causes the coacervate to form depends on the presence of electrokinetic potentials of opposite sign on the two reacting colloids, it is evident that the removal of electrokinetic potential on either colloid destroys the attracting force and likewise the coacervate. On a colloid carrying a negative charge, the zeta potential is most readily reduced by addition of polyvalent cations. A colloid carrying a positive charge is most susceptible to high valent anions.

In order for a coacervate to form, it is not necessary that the electrostatic forces of the two components be exactly balanced (Gortner, 1949). Figure 2 illustrates this diagrammatically. As negatively charged gum acacia is added to a positively charged gelatin sol, the viscosity of the mixture progressively decreases until at point B the coacervate begins to separate. Between B & C coacervate droplets retain a net positive charge, because the zeta potential of the gelatin which they contain is greater than that of gum acacia component. At C the electrokinetic potentials of the two components are exactly balanced, and the individual droplets are of themselves isoelectric, although they still contain both positively charged and negatively charged colloids. From C to D the potential on the gum acacia exceeds the potential of the gelatin, and the coacervate droplets as a whole possess

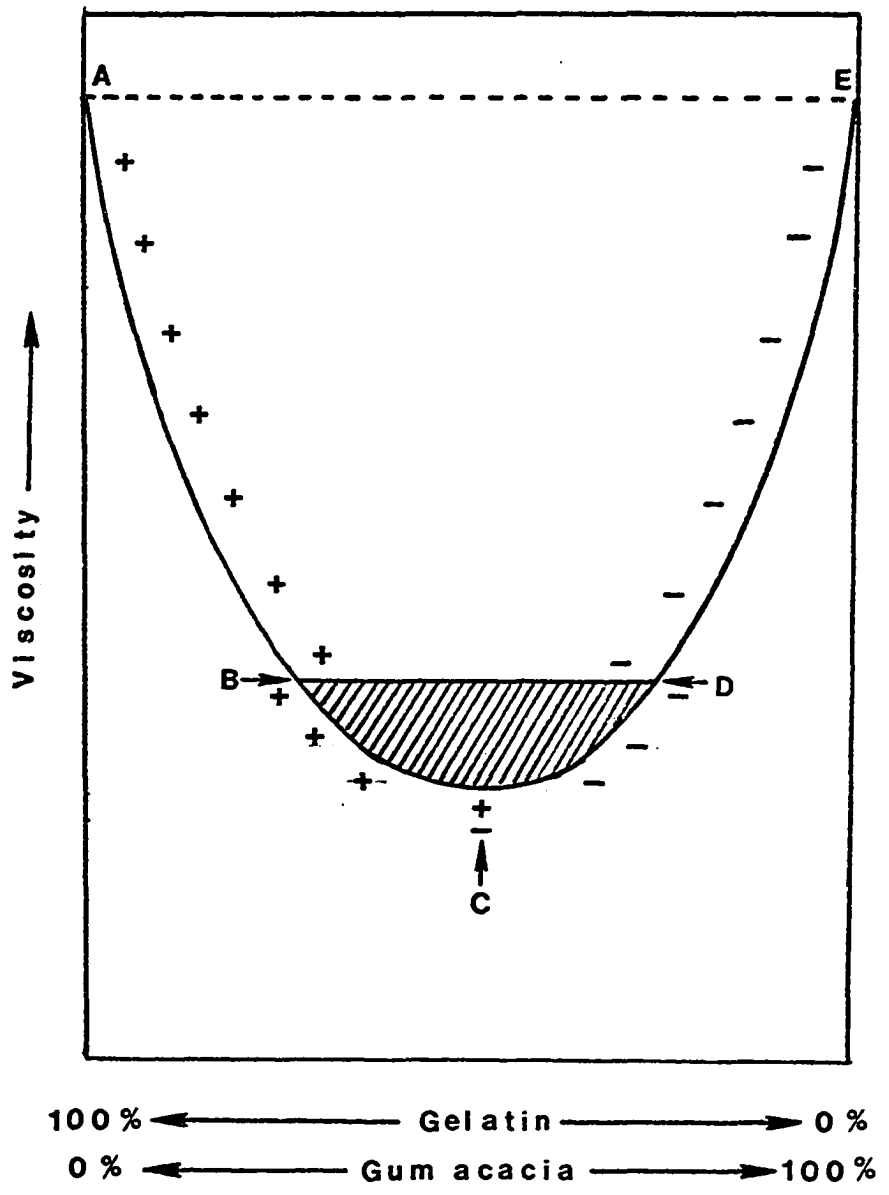


Figure 2. Relationship between pH and Electrokinetic Potential for Protein (A) and Non-Protein (B) Colloids.

an excess negative potential. In mixtures represented over the ranges AB and DE the electrokinetic charges and the kinetic energy of the individual colloids prevent the separation of a coacervate. The cross-hatched area of the diagram is the area within which a coacervate may be expected to separate.

Valency is one of the important factors involved in the behavior of ions toward a colloid system. In a series of papers, Hofmeister (1891) showed the effects of various anions and cations upon protein systems. Salts of monovalent ions, such as NaCl, act largely to reduce the zone of influence, ξ, of the charged particles. However, it has been noted that salts having divalent ions of charge opposite to that of the colloidal particle exert coagulation powers far beyond expectation. Salts having trivalent ions of opposite charge are even more effective. It was agreed by numerous workers that the anions can be arranged in a series of citrate> tartrate > SO_4 > acetate > Cl > NO_3 > Br > I > CNS, where, at least for protein systems, citrate shows the greatest precipitate effect and thiocyanate the least. Although, citrate and tartrate are more reactive with protein compared with sulfate, citrate and tartrate are considered toxic. Moreover, sulfate compounds are readily available and inexpensive. Therefore, the sulfated carbohydrate was chosen as a protein precipitating agent in this study.

Natural Carbohydrate Sulfates

Carbohydrate sulfates occur widely in nature. In the plant kingdom many algal polysaccharides belong to this group: the red seaweeds Chondrus crispus and Gigartina stellata contain carrageen poly-

saccharides. Dilsea edulis contains galactan sulfates, and the important polysaccharide agar-agar occurs in many species of Rhodophyceae. In the common brown seaweeds (Phaeophycae), the polysaccharide sulfate fucoidin is found. The animal carbohydrate sulfates found in nature include the chondroitin sulfate of cartilaginous tissue, the mucoitin sulfate of the gastric mucosa, and the blood-anticoagulant heparin (Percival, 1949).

With the above carbohydrate sulfate, carrageen is the most important natural polysaccharide sulfate in this study. The name carrageen was originally applied to the polysaccharide from species of Chondrus and Gigartina. The polysaccharides are extracted from the algae with hot water. They are normally isolated by precipitation from the aqueous extract by the addition of alcohol. Carrageens yield gels in the presence of certain salts, react with protein, and are precipitated by methylene blue. Carrageenan has been used as a thickening and emulsifying agent and as ingredient in food (Percival, 1949).

Carrageen has been used as a coagulant for pork waste and reduced COD and BOD 2,700 and 890 mg/L to 140 and 61 mg/L, respectively (Meada, 1974). A commercially available sulfated carbohydrate, Viscarin 402, is a refined water soluble carrageenan extracted from certain red marine plants (order Cigartinales) and reduced to a free-flowing powder by alcohol precipitation. It is composed primarily of sulfated D-galactose residues, linked together to form long chain polymers having molecular weights of several hundred thousand. It has the unique property of complexing and reacting with proteins. This property makes possible its effective use at very low concentrations. (Personal correspondence).

Sulfating Agents

Although carbohydrate sulfates are found in nature, they do not occur in appreciable quantities. The synthesis of carbohydrate must involve the reaction of sulfation, in which a new oxygen-sulfur bond is formed. Sulfating agents need to possess the properties and reactivities complementary to the formation of oxygen and sulfur bond. Several types of sulfating agents are discussed below.

Sulfuric Acid

Sulfuric acid has long been one of the most used industrial chemicals in the nation. It can be regarded as an extremely stable H_2O-SO_3 complex. Due to its ability to protonate electron rich centers, it has a high solvent power for organic compounds containing nitrogen or oxygen atoms. As a sulfating agent, concentrated sulfuric acid is a mild and cheap reagent and it is easier and safer to handle than other sulfating agents, such as chlorosulfonic acid, and SO_3. There are some disadvantages to using sulfuric acid as a sulfating agent, since sulfations are equilibrium reactions requiring an excess of reagents.

$$R\text{-}OH + H_2SO_4 \rightleftharpoons R\text{-}OSO_3H + H_2O \quad \text{- - - - - - - - - - - - (2)}$$

Sulfur Trioxide (SO_3)

Sulfur trioxide is the most powerful sulfating agent and reacts with almost all organic compounds. It exists in monomeric and several polymeric forms. SO_3 is monomeric in the vapor phase and as dilute solutions in SO_2, CCl_4, CH_2Cl_2, and other halogenated solvents. It is mostly trimeric in the liquid form, whereas the solid polymers consist

of chains of various lengths and degrees of cross-linking. The commercial use of sulfur trioxide depends on the addition of inorganic or organic stabilizers, such as borates and sulfonic acids, which inhibit polymerization and allow handling the material as liquid.

Undiluted liquid SO_3 reacts exothermically and so fast that charring occurs with all but the most stable organic compounds. Application as dilute vapors (up to 10 percent concentration) or in solutions of liquid SO_2 and other solvents avoids these difficulties. An alternate to dilution is to use sulfur trioxide in the form of its complexes. Combination of sulfur trioxide with various Lewis bases yields complexes whose reactivity depends primarily on the strength of the base. Suitable bases are ethers, tertiary amines, tertiary amides, thioethers, and tryalkylphosphates. Typically, they are somewhat soluble in organic solvents and deliver SO_3 to a reaction site in a controllable fashion. The complexes with trimethylamine, pyridine, dimethl formamide, dioxane, and triethyl phosphate are typical of those which have received considerable attention. Some of these complexes are described in more detail in a later section.

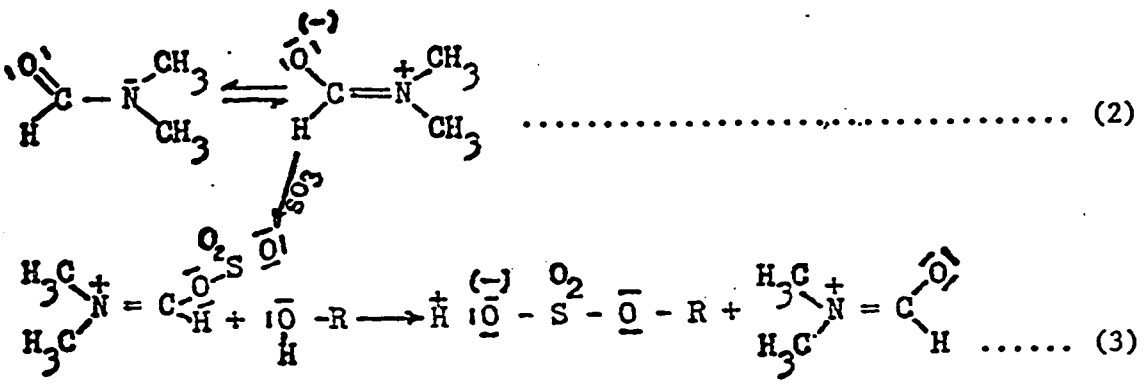
SO_3 has been widely used as a sulfating agent in commercial production because it is inexpensive and highly reactive. It has a higher use-efficiency than other sulfation agents. No excess reagent is required in sulfation when SO_3 is used. Unlike oleum, SO_3 is miscible in all proportions with liquid SO_2 and halogenated solvents. It can be introduced into the reaction as a solution. However, due to its high reactivity, more complex equipment and higher capital investment costs are required. In the uncombined form, SO_3 boils at 44.5° C., and melts at

168° C. However, it combines with itself to yield a cyclic trimer and various liquid and solid polymers. Because of its unique property, it is difficult to handle and must be kept dry, warm, and moving. Extreme care must be taken when SO_3 is used.

Sulfur Trioxide Complex

Sulfur Trioxide-Dimethylsulfate, (SO_3 - DMS). Mixtures of SO_3 and DMS contain dimethylpyrosulfate (CH_3OSO_2- OSO_2 - OCH_3). The only use has been in the production of 4, 4'-d-chlorodiphenyl sulfone, and 1-naphthalene sulfonic acid anhydride. Normally, two moles of SO_3 and one mole of DMS are mixed to give a solution of SO_3 in dimethylpyrosulfate. Since only one application has been described for the production of sulfones and sulfonic acid anhydrides, respectively, the scope and limitations of this agent are not well understood.

Sulfur Trioxide-Dimethylformamide, (SO_3 - DMF). Dimethylformamide is a very weak base and forms a poor and very reactive complex with SO_3. DMF is readily available and its complex with sulfur trioxide, as well as DMF itself, is quite stable. The stability of SO_3 - DMF complex in DMF solution is good; its efficiency does not decrease after four months at zero degrees C., even though the solution turns yellow and finally orange. Its structure, as suggested by Wolfrom and then Han (Schweiger, 1972), is as follows. The sulfur trioxide, with its electron-deficient sulfur atom adds onto the negatively charged oxygen atom of the polar mesomer of DMF. The sulfation presumably proceeds, as shown in the lower sequence - replacement of the DMF by the alcohol in a similar manner - with formation of the sulfuric ester and reformation of DMF.



(2)

(3)

Sulfur Trioxide-Trimethylamine (SO_3 - TMA). This is a very stable complex. The solid melts at 239° C. with decomposition and has a low solubility in organic solvents. The complex is quite stable in water solution, and has been used mostly for sulfations and sulfamation in this system. The complex can be prepared by combining SO_3 with TMA in the vapor phase or in solvents such as liquid SO_2 and dichloroethane.

Chlorosulfonic Acid (CSA). Chlorosulfonic acid is a strong acid. It dissolves in liquid SO_2 and in partially halogenated organic solvents. It freezes at -80° C., boils at 152° C. CSA, free or complexed, is used for the sulfation of alcohols (primary + secondary), ethoxylated alcohols, polyhydric alcohols, phenols and the sulfamation of aliphatic and aromatic amines.

Chlorosulfonic acid is a cheap, strong agent, but less destructive than SO_3. It has been largely displaced by sulfur trioxide in industrial applications because of the difficulties inherent in the disposal of the by-product HCl, but it remains an important laboratory reagent for sulfonation and sulfation, or when a sulfonyl chloride is the

desired product.

Methods of Sulfation

Carbohydrate can be sulfated by a variety of reagents. When D-glucose is dissolved in an excess of concentrated sulfuric acid below 0° C., a mixture of mono- and poly-sulfates is formed. The sulfation of sugar by concentrated sulfuric acid has been reported by several investigators in Japan and Europe. Takiura, et al. (1970) sulfated D-glucose with a tenfold volume excess of concentrated sulfuric acid at -5° C. A yield of 36 percent of 1, 3, 6 - D-glucosetrisulfate was reported. They also concluded that on the basis of an analysis of a reaction mixture by paper electrophoresis, 2.5 hours was the optimum reaction time. Shorter or longer reaction times do not favor the trisulfation.

Thorn and Simonsen (1970) prepared sugar sulfates by treating the saccharide with a double amount of concentrated sulfuric acid at a temperature of 0-15° C. They claimed that the preparation of sugar sulfates was dependent on the concentration of the sulfuric acid and the temperature of the reaction.

Nagasawa, K., et al, (1970) sulfated glucose by dissolving it in 96 percent sulfuric acid at 0-3° C. They used a mole ratio of 16:1 sulfuric acid to glucose. The products were separated through an ion exchange column, and finally recrystallized as monosulfate with ethyl alcohol.

Okuya, Takashi (1971) prepared a sulfated sugar by mixing the precooled concentrated sulfuric acid with sugar at a mole ratio of 10:1, H_2SO_4 to glucose at -25° C. for 2 hours. The glucose sulfate was isolated as a calcium salt.

Preparation of various cellulose sulfates using concentrated H_2SO_4 was reported by Ikumi Kagawa (1945). A barium salt of cellulose sulfate was prepared by dissolving cellulose in concentrated sulfuric acid, neutralization with $BaCO_3$, and addition of EtOH. Kagawa found that the reaction product polymerized very little and was subject to hydrolysis in the process. When the product was precipitated as a free acid, the yield was very low because of marked decomposition.

Sugar sulfate esters have also been prepared by using sulfating agents other than concentrated sulfuric acid. In the laboratory, however, the usual reagents for preparing sugar sulfates are chlorosulfonic acid in pyridine or the complexes formed by sulfur trioxide with such nucleophilic reagents as pyridine, N,N-dimethylformamide, trimethylamine and triethylamine. The efficiency of these sulfur trioxide complexes as sulfating agents for organic compounds has been reviewed (Turvey, 1965, Peat, 1960). Chlorosulfonic acid has certain disadvantages as a sulfating agent; it is unpleasant to handle, and generates chloride ions in the reaction mixture, but it is claimed to give mixtures of sulfates less complex than those afforded by the pyridine-sulfur trioxide complex. The sulfur trioxide complexes are relatively stable, and, with the commercial availability of a stabilized form of sulfur trioxide, are easier to prepare and to handle. Schweiger (1972) prepared sulfated cellulose using a complex of sulfur trioxide and N,N-dimethylformamide as the sulfating agents and claimed that the products had a degree of substitution greater than 2. He also reported that cellulose sulfates show a strong reactivity with proteins.

Ralph W. Kerr (1961) reported the method of sulfating cel-

lulose by treatment with a tertiary amine-SO_3 compound in contact with water and an alkali catalyst. Kerr also claimed that the use of an alkali catalyst was to promote the sulfation by opening up the lattices to allow entry to sulfating agent and by displacing the amine from the compound to form the alkali salt of the cellulose sulfate. The fibers were swollen by alkali and sulfated with triethylamine-SO_3 complex by maintaining a temperature 50° C in a water bath for 4 hours at a pH 11.1. The purified cellulose sulfate contained 0.51 percent sulfur.

In a U. S. patent, Leo H. Kruger and Otto B. Wurzburg (1969) reported the method for preparation of amylopectin sulfates using Me_3N-SO_3 as a sulfation agent. Amylopectin sulfates were prepared by reaction of amylopectin with Me_3N-SO_3 at pH 10.5-12.0, and a temperature of 122° F., and subsequently striping of the Me_3N. The total reaction time was 11 hours. A degree of sulfation of 1.4-2.0 was reported. Kruger found that failure to maintain the pH range or the use of vacuum and aqueous striping resulted in a markedly inferior product.

Ira B. Cushing and Edward J. Kratovil (1956) described the procedure for sulfation of polysaccharides using a mixture of dichloroethane and chlorosulfonic acid. Sulfation was carried out at 10° C. for two hours. After removal of the excess dichloroethane and chlorosulfonic acid, the reaction product was washed with dichloroethane, then with triethylamine, water, and iso-propyl alcohol. The syrup which formed was separated and washed with iso-propyl alcohol. The product was recovered as an Na salt.

Whistler (1967) sulfated cellulose and starch with a SO_3 and dimethyl sulfoxide (Me_2SO) complex, $(Me_2S{:}O)_x\ SO_3$ (x = 1-3), as a solid

or in solution in Me_2SO at ambient temperature. The reaction product was neutralized with aqueous alkali or ammonium hydroxides, carbonates or bicarbonates, and precipitated by the addition of a lower alcohol mixed with acetone. A degree of sulfation of 1.53 was reported for sulfated cellulose.

Sulfation of fatty alcohol with $ClSO_3H$ was reported in a German patent by Guiseppe Bozzetto (1961). A continuous sulfation process was carried out. Onto a rotating cone inside a reactor maintained at a constant pressure of 60 mm., a mixture of high fatty alcohol and $ClSO_3H$ was introduced continuously through 2 tubes in a ratio of 150:81. HCl was removed from the reaction vessel. The product was discharged continuously and neutralized with aqueous NaOH to give the Na salt of the sulfate. The degree of sulfation (D.S.) of the product was not reported.

Pyridine has commonly been used as a solvent for the carbohydrate and carbohydrate sulfate, but it has the disadvantage of being a poor solvent for simple sugar sulfates; furthermore, its subsequent removal from the products of reaction can be difficult. N,N-dimethylformamide has been used with success, as it is an excellent solvent for a great number of polymers, polysaccharides and their derivatives. Other systems that have been employed are concentrated sulfuric acid as the solvent, with chlorosulfuric acid as the sulfating agent. Sulfation of cellulose with concentrated sulfuric acid in the presence of an aliphatic alcohol and water was reported by Salam, et al. (1973). The results show that in the presence of n-butanol, a high degree of sulfation (D.S. 2.0) can be achieved. The product was completely soluble in water. The maximum sulfation was obtained with a cellulose to water ratio of 1:1. Water acts mainly as a swelling agent and, presumably,

optimum swelling was obtained at a cellulose/water ratio of 1:1. With larger proportions of added water, the dilution of sulfuric acid decreases its efficiency as a sulfating agent. A sulfation system using a mixture of concentrated sulfuric acid and ammonium sulfate in the presence of aliphatic alcohols and a procedure for preparing D-glucose 3-sulfate and D-glucose 6-sulfate using chlorosulfonic acid and pyridine-sulfur trioxide as the sulfating agents has been reported by Malm and Crane (1948).

Sulfating Conditions

Temperatere

Sulfation of a carbohydrate is temperature dependent. Different reaction temperature for sulfation was reported by several investigators, however, the optimum temperature was not determined. When concentrated sulfuric acid is used as a sulfating agent it is desirable for the reaction to take place at a low temperature to avoid thermal decomposition of carbohydrate. Four different temperature ranges, -5°C., 0-15°C., -25°C., and 0-3°C. have been reported in the literature (Takiura, 1970, Thorn, 1970, Okuya, 1971, Nagasawa, 1971). When SO_3 complex is used a higher temperature can be applied. Schweiger (1972) reported sulfation of cellulose with a DMF-SO_3 complex below 15°C. With a system of pyridine-sulfuric anhydride agent a temperature as high as 70°C. may be used. (Turvey, 1963).

Ratio of Carbohydrate to Sulfating Agent

The ratio of sugar to the sulfating agent can be varied depends on the material used for the sulfation. Different ratios of sulfating agent to carbohydrate has been reported in the literature; however in most cases

the sulfating agent was used in excess. Thorn and Simonson (1970) suggested a ratio of sulfuric acid to sugar of 2:1. Takiura (1970) dissolved D-glucose in tenfold volumes of concentrated sulfuric acid. Turvey (1963) reported that 5 grams of carbohydrate were treated with 3 moles of pyridine - sulfuric anhydride reagent.

Reaction Time

The time required to complete the reaction is important in determining the yield of the end product. A reaction mixture of glucose and concentrated sulfuric acid was examined by paper electrophoresis during different stages of the reaction. Takiura (1970) reported that the optimum reaction time for preparation of glucose trisulfate was 2.5 hours. Shorter or longer reaction time did not favor the trisulfation.

Type of Sulfating Agent

The choice of a sulfating agent and solvent also influence the complexity of the mixture of sulfates obtained from hexoses (Touey, 1963). Takiura, et al. (1970) reported that when using concentrated sulfuric acid as the sulfating agent as well as the solvent, three components mono-, di-, and trisulfate were detected in the reaction mixture during a certain period of time. Turvey (1963) reported that chlorosulfonic acid in pyridine at low temperatures afford the formation of the 6-sulfate. No other monosulfates and relatively few disulfates were detected. Pyridine-sulfur trioxide in pyridine gives more complex mixtures. When the latter reagent is used in N,N-dimethylformamide instead of pyridine, the mixture of products is somewhat less complex.

Methods of Identification

There are many different approaches used to isolate the sugar sulfates, the most common ones being paper chromatography, column chromatography, ion exchange, and dialysis. There is no method reported for the analysis of the free sugar acid. All the reported analyses used only sulfated sugar as a brucine salt.

Paper Chromatography

The method developed by Rees (1960) has been used by several different investigators. By using a system of butanol, ethanol and water (3:1:1 by volume; 100 ml) plus 3 g. of cetylpyridinium chloride, Rees separated five compounds after sulfating glucose with pyridine-sulfur trioxide at 65° C. A second system used by Rees was water-saturated methyl ethyl ketone (100 ml) plus 3 g. of cetyl pyridinium chloride. Although this system was less useful than the first, it resolved simple mixtures very rapidly. Silver nitrate-sodium hydroxide reagent, or p-anisidine hydrochloride were used to detect the location of the sugar in the above two methods.

Although paper chromatography will readily yield separation of reaction mixtures, the qualities which can be separated by this method are limited, and, therefore, attention has been focused on column chromatography and electrophoresis.

Paper Electrophoresis

Turvey, et al. (1963) followed the sulfation with pyridine-sulfuric anhydride by means of paper electrophoresis in neutral buffers. Turvey claims the products from galactose are thereby readily separated

into unchanged sugar, monosulfate, disulfate, and trisulfate in 2 hours with a potential gradient of 10 to 20 V/M in an acetic acid pyridine buffer (pH 6.5). Silver nitrate-sodium hydroxide reagent was used for detecting the sugar sulfate.

Takiura (1970) used paper electrophoresis to determine the progress of the sulfation of glucose with concentrated sulfuric acid. Different components of mono-, di-, and trisulfate were detected during the reaction. Takiura determined that 2.5 hours gave a maximum yield of trisulfate. A longer reaction time resulted in a hydrolysis of the trisulfate by the water formed. A neutral buffer of pyridine acetic acid (pH 6.5) was used and a potential gradient of 50 V/M was applied for 30 minutes in this system. The sugar sulfates were detected with aniline hydrogen phthalate. For the trisulfate, toluidine blue was also employed.

Column Chromatography

Chromatography on columns of cellulose powder has been used for separating the mixture obtained by sulfation of free hexose into unchanged hexose, hexose monosulfates, and hexose disulfates. Peat (1960) used ethanol, acetic acid and water (80:1:19) by volume to elute the unchanged sugar and monosulfate and then water to elute the disulfate.

Infrared

Infrared spectra have been used by several investigators for the characterization of carbohydrate sulfates. Lloyd and Dodgson (1964) noted that the strong absorption due to the S=0 bond at 1240-1250 cm^{-1} is general for all sulfate esters, but the bond in the region of 820-825 cm^{-1}, due to the C-O-S vibration, can be diagnostic for the position

of attachment of sulfate group. For D-glucose 6-sulfate, the peak appears at 820 cm^{-1}, whereas if a secondary hydroxyl group is sulfated, as in D-glucose 3-sulfate, the peak is at 832 cm^{-1}. The position of the peak at about 800 cm^{-1} was found to vary, depending on whether the ester was primary, secondary in an open chain, or secondary in a cyclic system. After an examination of the spectra of a number of polysaccharide sulfates, it was suggested (Lloyd, 1964) that the appearance of a peak at 850 cm^{-1} was due to a sulfate group occupying an axial, secondary position. It is thus established with reasonable certainty (Turvey, 1965) that sulfated sugars and sulfated polysaccharides will give an absorption band at about 1250 cm^{-1} and further bands in the region of 815-860 cm^{-1}, which can be diagnostic. For example, with hexoses, a peak at 810-820 cm^{-1} is characteristic of a 6-sulfate, with the sugar in the C-1(D) conformation; a peak at about 830 cm^{-1} can be attributed to a sulfate group occupying an equatorial, seconday position, and one at 850-860 cm^{-1}, to a sulfate group in an axial, secondary position.

Takiura (1970) used infrared spectra to determine the position of sulfate substitution. The infrared absorption bands at 810-820 cm^{-1}, 830 cm^{-1} and 850-860 cm^{-1} were assigned, respectively, to the sulfate C-O-S bond vibrations of primary, equatorial secondary, and axial secondary C-1 conformation. All of these positions unite to give an intense band at 800-810 cm^{-1}.

Schweiger (1970) has shown that the absorption of cellulose sulfate at about 3380 cm^{-1} and that of pure cellulose at about 3330 cm^{-1} definitely originates from bonded hydroxyl groups.

Periodate Oxidation

Periodate oxidation has been used by most investigators for determination of the structure of sulfated carbohydrates. It was assumed that the sulfate group does not interfere in the oxidation, and that, toward the periodate the ester group behaves as a simple blocking group (Turvey, 1965). For a free sugar the oxidation can proceed by way of either a ring form or the open chain form. When D-glucose 6-sulfate and D-galactose 6-sulfate were oxidized with an excess of sodium metaperiodate in unbuffered solution, the ratio of periodate consumed and formic acid liberated was about 3.5 moles per mole. In alkaline solution (pH 8) oxidation was more rapid, and the consumption of periodate approached 4 moles per mole. In contrast, Grant and Holt (1960) reported that D-galactose 6-sulfate consumes only 3 moles of periodate per mole, liberating 2.8 moles of acid.

The rate of hydrolysis of carbohydrate has been used by several investigators to diagnose the position of attachment of the sulfate group. Carbohydrate sulfate, like most esters, is labile to both acids and alkalis. Of particular interest in the study of carbohydrate sulfates is the rate at which sulfate groups are hydrolyzed under acid conditions and, stemming from this, whether differences in the rate of hydrolysis can be used for assigning positions to the sulfate groups. It is now well established that, in the cyclohexane system, ester groups at equatorial positions, and that sugar derivatives in the pyranoid form generally show a similar type of selectivity. Turvey (1965) reported that the rate of decomposition, by acid, of D-glucose monosulfates was in the order 3->4->6-sulfate. During a study of the rate of ester hydrolysis of

D-galactose 6-sulfate and D-glucose 3-sulfate, and of their methyl glycoside sulfates, it was found that the 6-sulfate is more stable than the 3-sulfate by a factor of three in the rate coefficient. Rees (1960) confirmed and extended these observations, showing that the rate of hydrolysis (in 0.25N hydrochloric acid at 100° C.) of sugar sulfates, and even of selected polysaccharide sulfates, could be diagnostic for the position of attachment of the sulfate group. In general, Rees could distinguish three groups of sulfates: (a) those with half-lives greater than 1.5 hours, in which the sulfate group was on the primary hydroxyl group; (b) those with half-lives in the region of 1 to 1.5 hours, in which the sulfate group was on a secondary, axial group; and (c) with half-lives in the range of 0.1 to 0.4 hours, all of these having the sulfate group on an equatorial, secondary hydroxyl group. Rees concluded that, for simple sugar sulfates with the possible exception of polysaccharides containing amino sugars and for homopolysaccharide sulfates, such studies could serve as a guide to the position of the sulfate group.

Turvey (1965) employed another system to separate the reaction mixture by using a series of solvents that selectively eluted the various sugar sulfates from a cellulose column. A solvent of 80% aqueous ethanol and 0.2% (V/V) of formic acid was used to elute glucose and monosulfate; a mixture of 60% aqueous ethanol and 0.3% formic acid eluted di- and trisulfates; and water was used to elute the tetrasulfate.

Schweiger (1972) employed dialysis techniques to separate cellulose sulfate from the reaction product due to the sulfation of cellulose with SO_3-DMF complex. The reaction product was dissolved and

dialyzed against distilled water for 48 hours. The dialyzate was concentrated to a small volume and the cellulose sulfate was precipitated by methanol.

Several investigators have utilized infrared spectra for the characterization of sugar sulfate. The C-O-S group vibration is near 850 cm^{-1}. Harris and Turvey (1969) have shown that this frequency is dependent on the phase the sugar sulfate was in, as well as the position of the sulfate substitution. For the infrared spectra three methods of preparing the sample were tested: KBr pellet, Nujol mull, and an aqueous solution evaporated to dryness on a silver chloride window. These samples were all handled as barium salts, and not as the free acids. The nature of the cation did not appear to affect the spectrum. The best results were obtained with the Nujor mulls. The band due to the S=O group at about 1205 cm^{-1} showed some variability, and could not be used for any diagnostic evaluations.

Mechanism of Sulfation

The sulfation reaction has been investigated by Eward (1976). The sulfation reaction can be divided into two classes;--addition to alkenes, and esterification of alcohols. The sulfation of carbohydrate may be regarded as esterification of alcohols. Eward reported that the reaction of alcohols with sulfuric acid proceeds by a bimolecular displacement mechanism like that of acid-catalyzed esterification. The observed rate expression is:

$$\text{Rate} = k\ \{ROH\}\{H_2SO_4\}\ \ (H^+ \text{ activity}) \text{ - - - - - - - - - - (3)}$$

Primary alcohols react an order of magnitude faster than secondary.

Since the reaction is an equilibrium process, optimization of yields depends on driving the equilibrium to the right by use of excess reagent or by removal of water. In the presence of sulfuric acid or sulfur trioxide, the dehydration of carbohydrate is less important than with secondary alcohols.

The reaction of an alcohol with sulfur trioxide may be considered as solvation of an anhydride.

$$ROH + SO_3 \longrightarrow ROSO_3H \quad (4)$$

Sulfur trioxide gives good yields of sulfates from primary alcohols, although the products are generally darker than those obtained by other reagents. Excessive dehydration occurs with long-chain secondary alcohols in the presence of sulfur trioxide.

The sulfation of alcohols by chlorosulfonic acid was also reported by Eward (1976). This is the most useful laboratory method.

$$ROH + ClSO_3H \longrightarrow ROSO_3H + HCl \quad (5)$$

The mechanism of the reaction parallels that of ester formation from alcohols. Because of the efficiency and high yields of the reaction, it also has been used in batchwise production of sulfates as well as in experimental continuous processes, but the corrosiveness of the by-product HCl makes it impractical for industrial scale production.

Protein Precipitation

Carbohydrate sulfate has been used to precipitate high molecular weight substances with basic groups. Such substances are: proteins, nucleic acids, and polypeptides. Jorgensen (1968) has developed a process to treat protein containing wastewater from food processing

plants by precipitation of protein, using sugar sulfate. By precipitation, 66-75 percent of the BOD_5, 18 percent or more of the phosphates, and 65-75 percent of the COD is removed. Furthermore, the suspended matter is reduced to approximately zero and the turbidity and color are reduced substantially (Jorgensen, 1968). The sludge obtained after precipitation, as well as the solution obtained by elution of the ion exchange columns, can be recovered as by-products and used as a food stuff. The value of the dried sludge removed from food processing wastewater was analyzed by Jorgensen. The amino acid balance of the sludge was determined. The results are summarized in Table 1. The amino acid content of the dried sludge from wastewater from a slaughterhouse, a herring filetting, a dairy, and a meat and bone meal production were compared with the need for the essential amino acids for chickens and swine. It can be seen from Table 1 that all dried sludges gave values acceptable for the application as a food stuff.

Thorn and Simonsen (1970) also used glucose polysulfate as a precipitating agent. They claimed that substances of high molecular weight having basic groups, such as protein, polypeptides, nucleic acids, or aminopolysaccharides, are precipitated from wastewater by addition of a 1 to 20 percent solution of mono- or poly-saccharide which is partially or completely esterified with sulfuric acid. Thorn and Simonsen (1970) also evaluated the precipitated product for use as food stuff. Their feeding tests were performed by direct feeding of precipitating agent test animals. The results showed that there was no weight loss; the food uptake was not deminished; no rise of transaminase activity was found; and urine retention or renal bleeding was not

Table 1. Amino Acid Analysis of Precipitated Sludge from Wastewater

	g Amino Acid per 16 g N.					
	I	II	III	IV	V	VI
Tyrosine	3.8	3.3	3.0	2.8	2.3	2.5
Phenylalanine	5.1	5.0	5.1	4.0	6.8	3.4
Methionine	2.3	1.4	1.2	1.9	0.8	1.3
Glutamic acid	14.1	20.3	6.6	10.8	10.5	12.8
Aspartic acid	9.7	8.3	8.7	8.4	11.0	7.6
Glycine	5.7	2.7	9.1	4.4	5.9	14.8
Alanine	6.1	4.0	7.0	5.7	8.1	7.2
Valine	6.4	6.5	6.6	5.4	7.8	4.1
Isoleucine	4.5	5.4	4.0	4.3	1.7	2.9
Leucine	9.6	9.5	9.2	7.0	12.4	6.2
Threonine	4.7	3.8	4.5	4.1	3.5	1.3
Lysine	8.0	6.6	7.0	6.1	8.7	5.4
Histidine	2.9	2.4	2.8	2.3	6.5	2.0
Arginine	6.2	3.9	6.5	3.2	4.9	6.4
Cystine	1.1	0.8	0	1.9	0.8	0.8
Tryptophane	1.7	-	-	0.9	1.4	0.2
Proline	4.6	8.1	6.5	3.1	4.1	8.6
Serine	4.3	3.8	4.0	3.5	4.1	3.8

I: Sludge from precipitation of waste water from slaughterhouse.
II: Sludge from precipitation of waste water from a dairy plant.
III: Sludge from precipitation of waste water from a meat- and bone meal factory.
IV: Sludge from precipitation of waste water from a herring filetting plant.
V: Blood albumin.
VI: Bone Meal.

observed. The product obtained from the precipitation of fish wastewaters with glucose pentasulfate was tested in chronic feeding experiments in rats. Thirty young rats were fed initially with 0.25 ml. of a 20 percent solution of glucose pentasulfate. The amount was increased after 3 weeks to 0.4 ml. glucose pentasulfate per day. All the animals developed very well and gained weight, and the female reats brought forth 10-12 living young. Both the parents before sexual maturity were raised in the chronic feeding experiments. They produced viable young, with no deformities. Thorn and Simonsen concluded that the product was harmless as a food stuff.

Various sulfate and sulfonate-related compounds have been used to precipitate protein from food processing wastewater. Lignosulfonic acid (LSA) is a by-product from the sulfite pulp and paper industry and is commercially available in a variety of forms. Therefore, LSA has been used by different investigators as a protein precipitating agent.

If the process requires recovery of the precipitated protein sludge as by-product, which is the case in this study, it would be disadvantageous to use lignosulfonic acid as a protein precipitating agent. Lignosulfonic acid precipitated protein sludge is toxic as an animal feed. In order to compare the effectiveness of protein removal by carbohydrate sulfate and other available protein precipitating agents, various systems which utilize LSA as precipitating agents are presented.

The physical-chemical combination of lignosulfonic acid and protein in an aqueous system has been known for over 30 years (Gustavson, 1942, Wilson, 1943). Wallerstein (1944) described the recovery of pro-

tein from dilute solutions, using spent sulfite liquor at a pH of 2 to 4. The precipitation agent in the sulfite liquor was identified as lignosulfonic acid and a ratio of 2 parts lignin to 5 parts protein was observed to be optimal.

Tonseth and Berridge (1968) reported the results from a pilot plant study in which lignosulfonic acid was used to precipitate protein from various industrial wastewaters. Removal of 60-88 percent of the BOD, 73-86 percent of the COD, and 17-95 percent of the organic nitrogen from a slaughterhouse waste was reported.

Pearl (1957) discussed the uses of lignin in a survey paper in 1957, and mentioned the reaction of lignosulfonic acid with protein to form insoluble complexes, and indicating that this reaction can be used to remove protein from effluents of canneries or fish-processing plants.

A method of purifying protein-containing liquids was patented by Lief Jantzen of Norway (1968). Lignosulfonic acids were added to the protein-containing liquid to promote the precipitation of protein-lignosulfonic acid and separation by dissolved air floatation.

Several patents based on Norwegian investigations describe a process for treating meat-packing wastes based on either the use of salts and mineral acids to reduce the pH to 2 and precipitate the complex, or the use of sulfuric acid ester of sugars, such as sucrose or lactose, as precipitants (Nettli, 1974, Aktieselskapet, 1974, Asker, 1974). In the latter case, esters having a molecular weight equal to or greater than 200 gave BOD reductions as high as 80 percent, depending on dosage. The precipitated material could not be used as cattle feed.

The lignosulfonic acid (LSA) process has been used successfully

by several investigators for treating various food processing wastewaters in both pilot plant and demonstration studies. (Jorgensen, 1968, Tonseth, 1968, Felicetta, 1971, Rosen, 1971, Naabye, 1972, Hopewood, 1972, Foltz, 1974, Crocco, 1975). BOD, TSS, oil and grease, and nitrogen contents were lowered markedly, and proteins and fats were recovered by adding LSA to the wastewater at pH 3, followed by air flotation to separate the lignoproteins. The resulting sludge had a protein content of up to 52 percent, and an amino acid profile that compared favorably with that of soybean meal and casein. Results of the studies are summarized in Table 2.

Claggett (1972) presented details of the design and operation of a chemical treatment and air flotation system for treating fish processing wastes at Steveston, B. C., Canada. He found that lignosulfonic acid treatment gave a floc that was fragile and not easily removed. He further reported that alum-caustic ($Al_2 (SO_4)_3$ --NaOH) treatment at pH 9.2, followed by readjustment of the pH to 5.2, resulted in a curdy floc that floated readily. COD, protein, insoluble solids, and soluble solids removals of 84, 61, 92, and 28 percent, respectively, were obtained by this process.

Aktieselskapet Apothekernes Laboratorium (1969) invented a process by which protein contained in wastewater can be precipitated at a pH of 3.5, using sulfonates or sulfates of fats, fatty oils, fatty acids, or fatty alcohols. The preferred precipitating agent was sulfated C_{8-20} fatty alcohols. Precipitation depends on the molecular weight and chemical characteristics of the protein.

Table 2. Summary of the Removal Efficiency of Lignosulfonic Acid Process

Investigators	Waste	Removal Efficiency (%) BOD	COD	Org-N	TSS	O&G
Tonseth & Berridge	slaughterhouse	60-88	73-86	73-95	76-90	---
Jorgensen	slaughterhouse	58	56	52	---	---
Felicetta & Peacock	fish	---	---	94	---	---
Rosen	poultry	80	---	---	---	---
Fultz et al.	slaughterhouse	84	---	87	96	95
Hopewood & Rosen	slaughterhouse	88	75	95	82	---

CHAPTER III

EXPERIMENTAL PROCEDURES

Starting Material

Pure anhydrous glucose, corn syrup, Whatman cellulose powder CF-II, Whatman filter paper, newsprint paper, kraft paper, wheat straw, beef fat, fatty alcohol, and lard were tested for use as carbon sources to prepare sulfated carbohydrates with concentrated sulfuric acid and DMF-SO_3 complex. All the samples were air-dried. When DMF-SO_3 complex was used the carbon source materials were swollen with DMF to increase the accessibility. Usually, sample carbohydrates (10g) were mixed with one hundred fifty ml. of DMF in a Waring blender and allowed to stand in the blender for 2 hours. Two different grades of DMF were used in the experiment. Reagent grade of DMF was used in the preparation DMF-SO_3 complex. Technical grade of DMF was used in swelling the carbon source material.

Preparation of Protein Wastewater

Standard protein wastewaters were prepared to evaluate protein removal efficiency using sulfated carbohydrates. Several materials were tested to determine the applicability of standard protein wastewater. One of them was an egg-white solution. A dried egg-white powder was prepared by freeze-drying, in which water was removed under a vacuum and low

temperature. A standard egg-white solution was prepared by dissolving a controlled amount of egg-white powder in cold water. The egg-white solution was very stable upon adjustment of pH.

Another protein wastewater solution was prepared by blending hamburger beef in a blender. After a series of tests it was determined that five g. of beef blended with five hundred ml. of water for one minute gave a wastewater with the strength comparable to a typical meat-packing plant effluent waste stream. Ground beef meatballs with a weight of five g. were prepared from the same batch of beef and stored in the freezer. Sodium caseinate could not be used as a protein source, because it precipitated upon acidifying at the pH of 5.

Sulfation with Concentrated Sulfuric Acid

Pure glucose or cellulose was added to cooled, concentrated sulfuric acid with different mole ratios of glucose or cellulose to sulfuric acid and stirred for the desired period of time at a selected temperature. The reaction temperature was controlled, not to exceed 7° C. After the reaction was completed, the reaction mixture was poured into ice and then diluted with deionized water.

Sulfation with DMF-SO_3 Complex

Sulfation was carried out by mixing stabilized sulfur trioxide with N, N-Dimethylformamide (DMF). One thousand ml. of reagent grade DMF was placed in a 3-necked, round-bottom flask equipped with a mechanical stirrer, a $CaCl_2$ drying tube, and a two hundred fifty ml. dropping funnel. The reaction was exothermic; therefore the flask was cooled in an ice bath in order to maintain the temperature below 40° C. Sulfur tri-

oxide (30g) was added dropwise into the flask during the 1-1.5 hours. The DMF-SO_3 complex was obtained as a yellowish, crystalline slurry. This mixture of complex and DMF was stored under refrigeration and used in the sulfation.

Ten grams of carbohydrate, dried for 2 hours at 105° C., were mixed with one hundred fifty ml. of technical grade DMF, kept for 2 hours at room temperature, and blended in a Waring blender for 2 minutes. Then the carbohydrate slurry was transferred to a 3-necked, round-bottom flask and immersed in a circulated cold-water bath. Fifty g. of DMF-SO_3 complex was added to the slurry while mixing. The temperature was maintained below 35°C. throughout the reaction. The reaction time varied from 1 to 3 hous. When the reaction was completed, a gel type raw product was obtained.

The sodium salt of the sulfated carbohydrate was prepared by neutralizing the raw product with dilute sodium hydroxide. After filtering through a Buchner funnel to separate the unreacted paper, the filtrate was concentrated to 20 percent of the original volume under vacuum. Then the concentrated solution was placed in several cellulose dialysis tubes and dialyzed with distilled water for 2 days. After a negative test for free inorganic sulfate with barium hydroxide, the volumes in the dialysis tubes were reduced to 20 percent of their original volume under vacuum. The sodium salt of the sulfated carbohydrate was precipitated by pouring the solution slowly into two volumes of ethyl alcohol. After filtering and drying in an oven at 80°C., the product became a crystal which readily dissolved in water.

The method used to refine the product into sodium salt is expensive and time-consuming. It involves a process, such as electrodialysis, to remove unreacted inorganic sulfate and DMF. Moreover, the yield of the product relative to the raw starting material is only 2 percent, if the product is recovered as a sodium salt. Therefore, the product must be recovered as a dried acid form. Recovery of the final product in dried acid form requires removal of the DMF from the raw product. DMF is an organic solvent which has a high COD. If DMF is not removed from the raw product, the treated effluent often has higher COD than the raw waste. DMF is a very polar solvent which can easily bind to another polar or ionic molecule such as sulfated carbohydrate. The DMF can be separated from the raw product by washing with a non-polar solvent, such as ethyl alcohol. The washing process is carried out by blending and centrifuging the raw product with ethyl alcohol.

The washing process consisted of mixing 10 grams of raw product in a Waring blender with 150 ml. of ethyl alcohol for one minute; then the mixture was transferred to several centrifuge tubes and centrifuged at 5,000 rpm for 10 minutes. The alcohol and DMF mixture was collected and the solids transferred to a small blender. A similar washing process was repeated 3 times. After the washing process was completed, a DMF free final product was obtained and the solids were dried in an oven at 80° C. for two hours.

The ethyl alcohol and DMF mixture, which was collected from the washing processes of the refined raw product, was separated by distillation. About three hundred to three hundred fifty ml. of ethyl alcohol was used in refining 10 g. of raw product. The collected alcohol and

DMF mixture was distillated with a conventional distallation set-up. The mixture started to boil at 75° C., and the distillate is collected from that temperature to 135° C. The portion collected between 75 to 90° C. is mainly alcohol, which is composited and reused in the subsequent cycle of washing. The portion of the distillate which collects between 90 to 135° C. is a mixture of alcohol and DMF. Further separation of alcohol and DMF is not carried out. A small portion of the Distillate collects above 135° C. is DMF. The amount of DMF collected from each distillation is insufficient to reuse in another run of sulfation. While the amount of DMF recovered in the laboratory is negligible, recovery of DMF will be feasible on an industrial scale.

Determination of Protein Removal Efficiency

The beef waste was prepared by blending 5 grams of hamburger beef with 500 ml. of distilled water for one minute, then filtered through a piece of paper towel. A 0.05 percent egg-white solution was prepared by dissolving dried egg-white powder in distilled water. Five hundred ml. of the protein wastewater was adjusted to a pH of 3.5 to 4.5 with dilute sulfuric acid, and placed under a bird stirrer. After addition of the desired amount of sulfated carbohydrate, the protein wastewater was rapidly mixed (100 rpm) for two minutes; then slowly mixed for 15 to 20 minutes to allow the floculation to occur. The precipitate was allowd to settle for 2 hours. The COD of the untreated protein wastewater and the treated effluent, which was taken from the top of the settled solution, was determined. The removal efficiency was calculated, based on the COD before and after the treatment.

Product Identification

A few mg. of purified product was mixed with about one hundred mg. of dried potassium bromide powder in a small mortar. Then it was transferred and pressed in a special die to yield a transparent disk. The disk was then held in the infrared instrument for spectroscopic examination.

Determination of Degree of Sulfation

The sample (0.5 - 1.0 g.) was refluxed in two hundred fifty ml. of a 10 percent hydrochloric acid solution overnight, and the sulfate was precipitated by the addition of twenty-five ml. of a 10 percent barium chloride solution. The precipitate was transferred to a tared Gouch crucible, heated one hour at 300° C., and ignited for one hour at 600° C. The sulfur content and degree of sulfation of the sample were calculated as follows:

$$\% S = \frac{(0.13737)\ (\text{wt. of barium sulfate, g})}{(\text{sample wt., g})}$$

Degree of sulfation (based on the sodium salt) (D.S.)

$$D.S. = \frac{(1.62)\ (\% S \text{ in sample})}{32 - (1.02 \times \% S \text{ in Sample})}$$

CHAPTER IV

RESULTS AND DISCUSSION

Methods for preparation of sulfated carbohydrates using corn syrup, kraft and newspring paper have been developed. The sulfated carbohydrates were used as a precipitating agent to remove proteins and suspended solids from protein containing wastewater. The removal efficiency of proteins was evaluated by the determination of the chemical oxygen demand (COD) before and after chemical treatment. The experimental conditions and data are given in Appendix A. The development of methods and experimental resultas are discussed below.

Selection of Sulfation Agents

Several sulfation agents--concentrated sulfuric acid, fuming sulfuric acid, chlorosulfuric acid, sulfur trioxide and a complex of dimethylformamide with sulfur trioxide, were considered for this study. Preliminary screening tests were performed to determine the most suitable agents.

Based on the preliminary screening results and the literature review, concentrated sulfuric acid and a complex of dimethylformamide with sulfur trioxide were selected as sulfation agents. The characteristics and effectiveness for sulfation using these two chemicals and or their complexes were discussed.

As a sulfonating-sulfating agent, concentrated sulfuric acid is a mild, cheap reagent. It is easier and safer to handle than chlorosulfonic acid or SO_3. Concentrated sulfuric acid has been successfully used in the laboratory to prepare sulfated sugar (Takiura, 1970, Thorn, 1970). In the preliminary test, the mixture of corn syrup and concentrated sulfuric acid is an extremely stable compound, very easy to store, and does not require any special equipment to transport. The disadvantage of concentrated sulfuric acid is that sulfation is an equilibrium reaction requiring an excess of reagent. In order to prevent thermal degredation of the sulfated sugar, it is necessary to store the product in a dilute solution. High cost of storage and transporting of the product is another disadvantage of using concentrated sulfuric acid as sulfating agent.

Sulfur trioxide is another sulfating agent chosen for this study. Sulfur trioxide was the most powerful sulfonating-sulfating agent tested. It reacts with almost all organic compounds; has a high use-efficiency; and excess of reagent is not required. Due to its unique physical properties and high reactivity, extreme care must be taken when sulfur trioxide is used. The ideal condition is to store the sulfur trioxide at a constant temperature, between 35-43° C.

Direct sulfation of carbohydrate with sulfur trioxide is not feasible. Sulfation is usually carried out with sulfur trioxide in a solvent. Sulfur trioxide can complex with some nucleophilic reagents, such as trimethylamine, triethylamine, poly(2-vinyl pyridine), pyridine, N,N-dimethylformamide, and dimethylsulfoxide to form a stable compound

by which sulfur trioxide was introduced to initiate the sulfation reaction.

Based on the results of the literature review, N,N-dimethylformamide was selected and sulfur trioxide complex was selected as the sulfation agent. Both DMF and SO_3 are commercially available. The SO_3 and DMF complex is very stable. After the complex is formed, it can be stored for a long period of time without degradation. The complex has only a slight effect on the decomposition of polysaccharide macromolecules during the course of sulfation reaction. Moreover, the DMF itself is an excellent solvent for many polymers, polysaccharides and their derivatives. An advantage of the sulfation process involving DMF is that the sulfated carbohydrate formation is not accompanied by the liberation of water molecules, so that no free sulfuric acid can be formed, making it possible to prevent the hydrolytic degradation when the sulfated carbohydrate is stored.

Selection of Carbohydrate

Anhydrous glucose has been used in the laboratory as the starting material to prepare sulfated sugar. Glucose is not the most readily available substance in the biosphere, from an economic standpoint, and, therefore, is not suitable for use as the starting material in large-scale production. One of the objectives of this study is to develop a method of sulfation of carbohydrate, using an inexpensive starting material.

The sulfation process converts a pure alcohol to its sulfate or a mixture of fatty alcohols to their sulfates. Therefore, by definition, the starting raw material must possess at least one OH functional group. In addition, an organic solvent must be able to swell a starting material. Wheat straw, newsprint paper, kraft paper, corn syrup, molasses, cornstarch, beef fat, lard, pure cellulose powder, filter paper and glucose were

evaluated as starting materials.

Preliminary tests were performed to determine the feasibility of each raw material before a detailed study was performed. Each of the raw materials was treated with an equal weight of DMF-SO_3 complex for two hours at room temperature. A two percent mixture of raw product was used to treat beef-processing wastewater. The results indicated that the wheat straw produced the lowest COD removal efficiency, and therefore, was eliminated from further study. Beef fat and lard have a very low solubility in DMF; therefore sulfation was not possible, so they were eliminated as starting materials. The sulfated corn syrup, molasses and cornstarch showed good removal efficiency of protein from wastewater. However, these materials are more expensive than newsprint and kraft paper. The results indicated that by using DMF-SO_3 as sulfating agent, the COD removal efficiency using sulfated newsprint and kraft paper is comparable with sulfated corn syrup and molasses. The cost to prepare a DMF-SO_3 complex is higher than the cost of concentrated sulfuric acid. To use DMF-SO_3 complex as sulfating agent, a less expensive carbohydrate needs to be chosen to minimize the total cost of starting materials. Newsprint paper and kraft paper is readily available and is less expensive than corn syrup and molasses. Therefore, from an economic viewpoint, newsprint paper and kraft paper would be a better choice for starting materials. According to a survey, performed in 1968 by APWA, the current rate of solid waste generation in the United States is about 5.3 pounds/person/day, or roughly 185 million tons/year. About 40-60 percent of the municipal waste generated in the United States is paper material (APWA, 1970). By using newsprint and kraft paper as starting materials, one

form of waste is being used to treat another.

Method of Development

This study was divided into two phases--sulfation with concentrated sulfuric acid and sulfation with a DMF-SO_3 complex. In this section the data from two phases are presented and the results compared.

Sulfation with Concentrated Sulfuric Acid

Anhydrous glucose and concentrated sulfuric acid were used as starting materials at the beginning of the study. An acid-to-sugar ratio of 2 to 1 was used. The glucose and sulfuric acid were mixed for two hours at between 5-7° C. The reaction products (20 percent dilution) were used to treat the meat-packing wastewater. The COD removal efficiency is presented in Table 3. It is difficult to mix an anhydrous powder with a highly viscous liquid such as concentrated sulfuric acid. Homogenous mixing has never been achieved, either by manual or automatic feeding. At the end of 2 hours of mixing by a mechanical stirrer, some glucose powder lumps covered by concentrated sulfuric acid were observed. It is apparent that the granular glucose anhydrous is difficult to incorporate into a large-scale processing plant with the same ease and efficiency of a liquid reactant.

A liquid reactant such as corn syrup was utilized as a starting material to prepare sulfated sugar. A commercial-grade corn syrup was mixed with concentrated sulfuric acid under the same conditions as those for preparing sulfated glucose. The liquid reactant mixed well with the sulfuric acid during the first 30 minutes; afterward, some corn syrup droplets began to appear. This phenomena continued through the end of the reaction. After 2 hours of mixing, the reaction product was diluted

Table 3. COD Removal Efficiency of Beefwaste with Sulfated Glucose*

Sample No.	COD Before Treatment mg/L	COD After Treatment mg/L	Removal Efficiency %
CS 1	5396	676	87.4
CS 2	5396	1056	80.4
CS 3	5396	615	88.6
CS 4	5396	539	90.0
CS 5	5396	725	86.6
CS 6	5396	824	84.7
CS 7	5396	794	85.2
CS 8	5393	778	85.6

*Using Concentrated Sulfuric Acid as Sulfating Agent

to a 20 percent solution and used to precipitate proteinaceous waste. The COD removal efficiency is given in Table 4.

Less expensive sugar sources, such as molasses and starch, were also used as starting materials to prepare sulfated sugar under the same conditions as described previously. Since the color of molasses is dark, it was not possible to distinguish the unreacted sugar simply by observation. The results of treatment of proteinaceous waste with sulfated molasses are presented in Table 5.

The results in Tables 3, 4 and 5 show that sulfated corn syrup has the highest COD removal efficiency among the three sulfated sugars. The average COD removal efficiency is 88 percent, 86 percent and 76 percent for sulfated corn syrup, glucose, and molasses, respectively.

Newsprint paper and kraft paper were sulfated with concentrated sulfuric acid with an acid-to-paper ratio of 2:1 for 2 hours. After filtering to remove the paper fiber, the filtrates were used to treat proteinaceous waste, and no precipitate was observed. In order to promote the sulfation of newsprint paper and kraft paper with concentrated sulfuric acid, attempts were made to increase the contact surfaces between the acid and paper. Newsprint and kraft paper were blended with water, then dried in an oven prior to mixing with sulfuric acid. However, increasing contacting surface did not promote the sulfation. After increasing the acid-to-paper ratio from 2 to 6 and extending the reaction time from 2 to 4 hours, the sulfated paper gave only 45 percent of COD removal. The concentrated sulfuric acid is a mild sulfating agent for the carbohydrate with the long chain structure, such as newsprint and kraft paper. In addition, the paper fiber not being properly swollen by an organic

Table 4. COD Removal Efficiency of Beefwaste with Sulfated Corn Syrup*

Sample N.	COD Before Treatment mg/L	COD After Treatment mg/L	Removal Efficiency %
CS 9	5396	805	85.1
CS 10	5396	524	90.3
CS 11	5396	380	92.9
CS 12	5396	539	90.0
CS 13	5396	737	86.3
CS 14	5396	842	84.4
CS 15	5396	961	82.2

*Using concentrated sulfuric acid as sulfating agent

Table 5. COD Removal Efficiency of Beefwaste with Sulfated Molasses*

Sample N.	COD Before Treatment mg/L	COD After Treatment mg/L	Removal Efficiency %
CS 17	5975	1470	75.4
CS 18	5975	1356	77.3
CS 19	5975	1906	68.1
CS 20	5975	1505	74.8
CS 21	5975	1600	73.2

*Using Concentrated Sulfuric Acid as Sulfating Agent

solvent may be another reason for the sulfation not taking place.

The sulfated carbohydrates prepared with various sugars under varying experimental conditions were used as precipitation agents to remove protein and suspended solids from meat-processing wastewater. The optimum experimental conditions and chemical dosages were also determined.

Bench scale tests were conducted to determine the protein removal efficiency for each sulfated carbohydrate. Preliminary jar tests were performed to determine the chemical dosages. Results were obtained by visual inspection of the precipitation and settling characteristics. The pH measurements were recorded to study the effects of pH on protein precipitation, and pH control was found to be critical. pH is a function of the amount of chemical added; an increase in the dosage decreases the pH. The net charge on the protein is pH-dependent. Protein is least soluble at its isoelectric point. The isoelectric point varies with different proteins. Generally, acidification to pH 4.9 or below altered the isoelectric structure of the soluble proteins and aided in the separation and coagulation of the suspended matter. A 20 percent solution of sulfated glucose prepared with concentrated sulfuric acid was used to determine the chemical concentration required for optimum protein precipitation. The results are shown in Table 6.

Table 6 reveals that good to excellent precipitation occurred in the pH ranges 3.8-5.0. Experimental evidence confirmed that dosage control is important in the treatment of proteinaceous waste with sulfated carbohydrates. The relationship between COD removal efficiencies and different chemical dosages of sulfated glucose, sulfated corn syrup, sulfated molasses and sulfated starch is shown in Figs. 3 through 6,

Table 6. Effect of pH and Dosage on Protein Precipitation with Sulfated Sugar.

Dosage ml	pH Range	Precipitation
Above 0.80	0-2.0	Poor
0.50-0.60	2.8-3.7	Fair
0.20-0.40	3.8-5.0	Good
0-0.12	Above 5.5	Poor

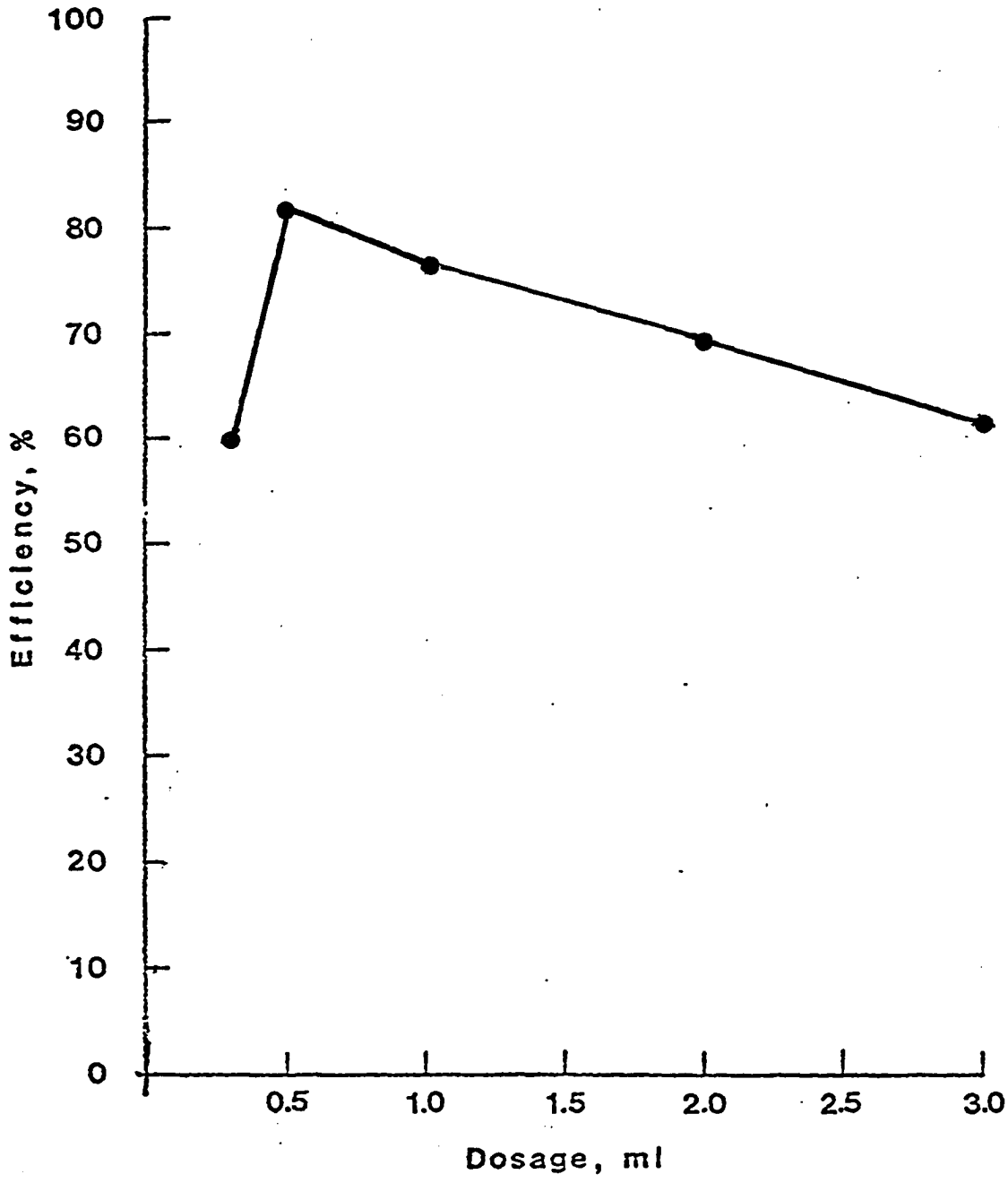


Figure 3. Efficiency in the Removal of COD by Addition of Different Amounts of Glucose Trisulfate.

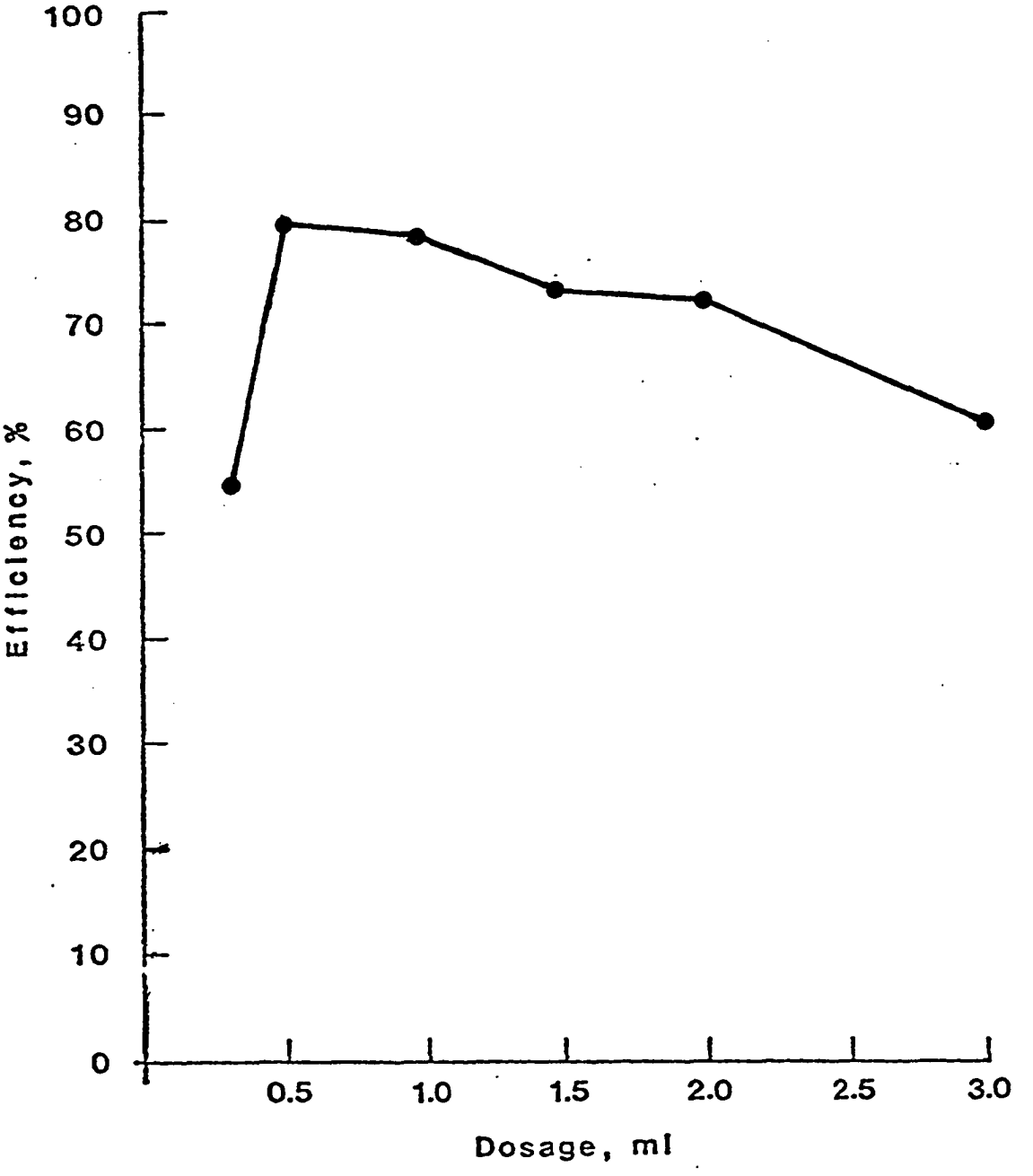


Figure 4. Efficiency in the Removal of COD by Addition of Different Amounts of Corn Syrup Sulfate.

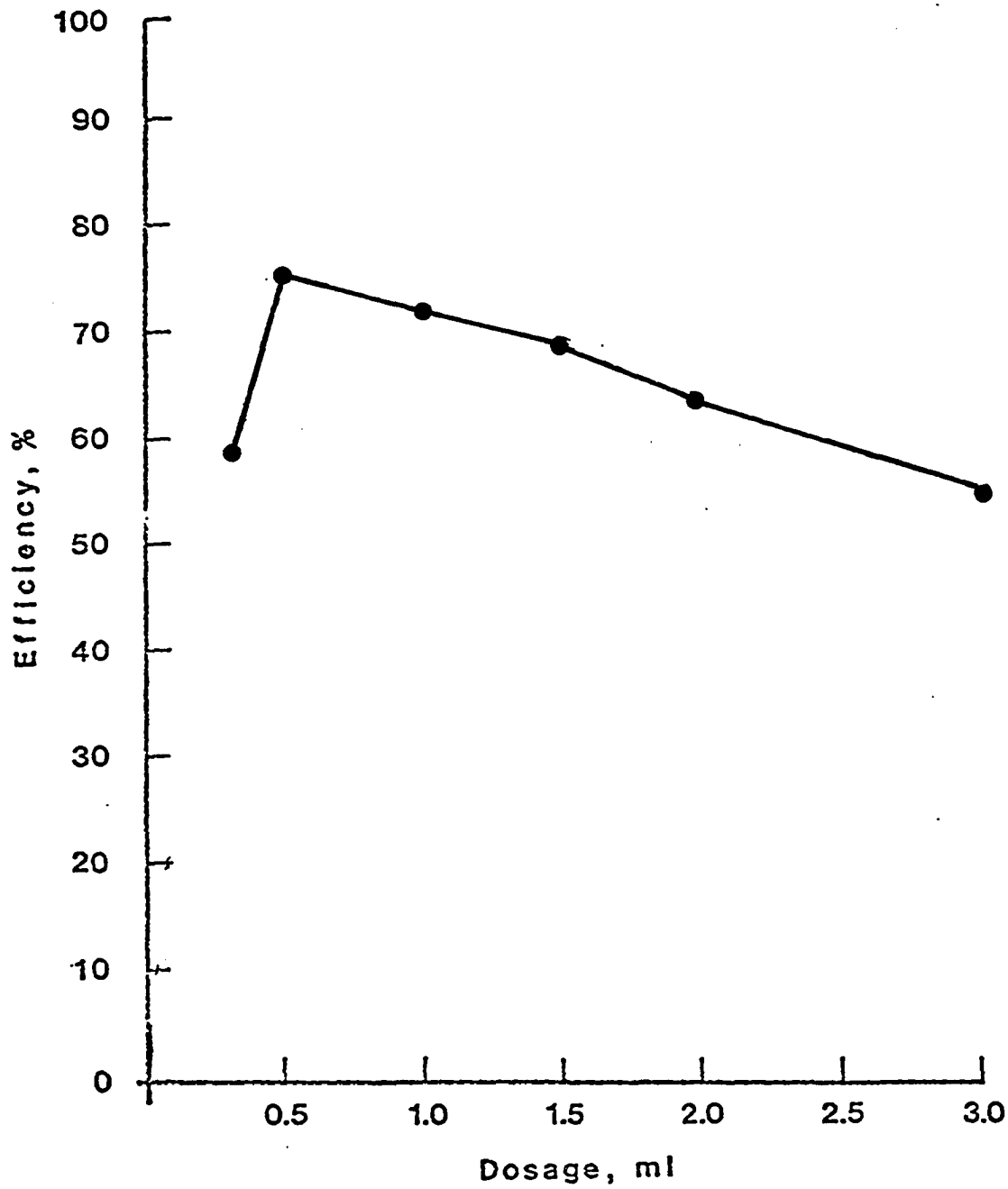


Figure 5. Efficiency in the Removal of COD by Addition of Different Amounts of Molasses Sulfate.

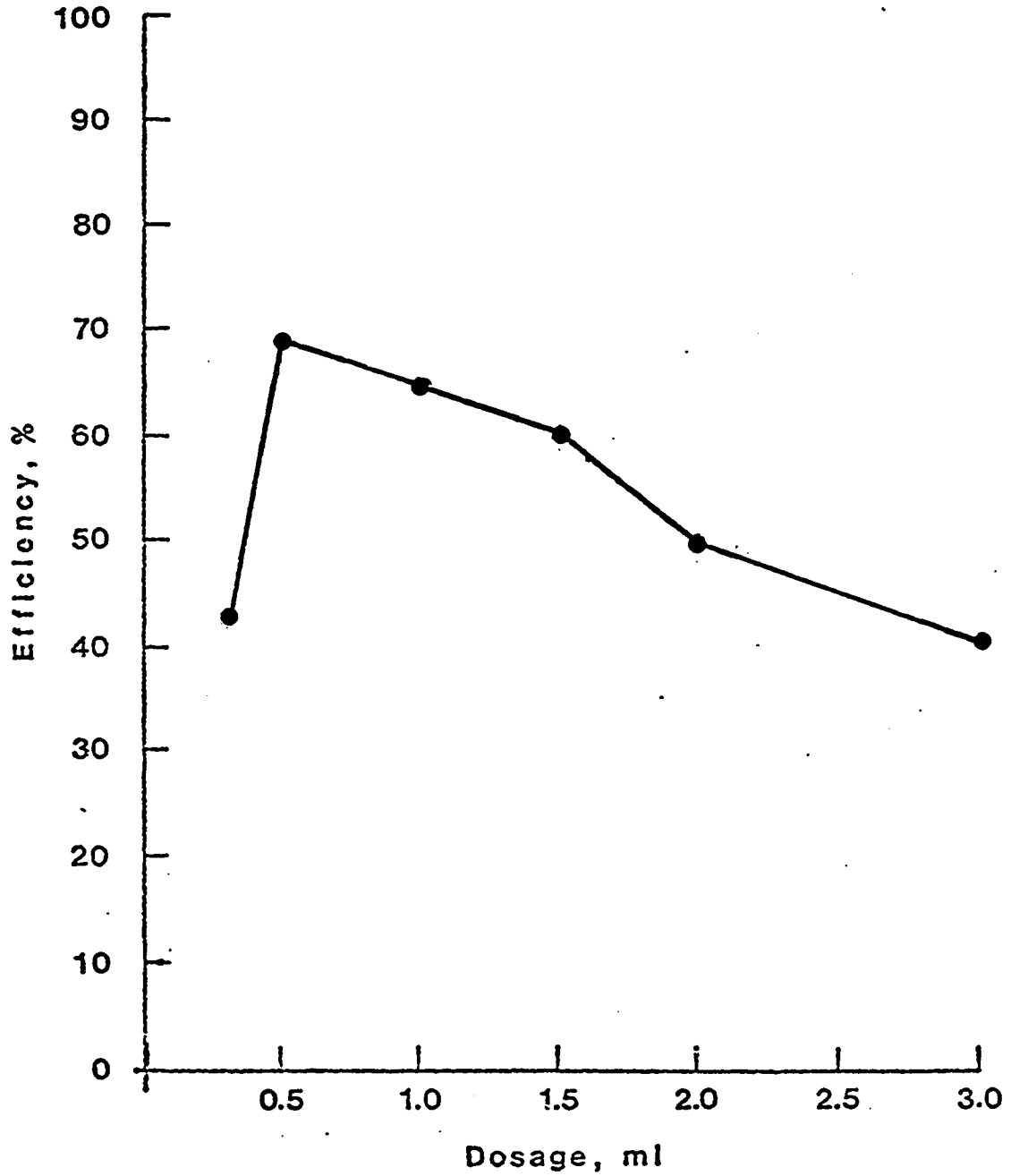


Figure 6. Efficiency in the Removal of COD by Addition of Different Amounts of Starch Sulfate.

respectively. The chemical analyses of the effluent treated with sulfated carbohydrate are compiled in Appendix A, Table A10 to Table A13, respectively.

From Figs. 3 through 6, it can be seen that the maximum COD removal is achieved at the optimum dosage of 0.5 ml. (0.20 g/l) within the pH range of 3.8-5.0 for all four of the sulfated sugars. The COD removal efficiencies for sulfated glucose, corn syrup, molasses and starch were 82, 80, 76 and 69 percent, respectively. The protein precipitation efficiency was higher for sulfated glucose and corn syrup than for sulfated molasses and starch. Increasing the chemical dosage above the optimum did not improve floc formation, but decreased the removal efficiency of organic matter and increased the turbidity in the final effluent. It was speculated that the floc which was initially formed may undergo hydrolysis in the low pH solution.

Sulfation with DMF-SO_3 Complex

Sulfur trioxide is a powerful sulfation agent, but cannot be applied directly to react with carbohydrate due to its high reactivity. It must be complexed with a nucleophilic reagent to form a stable compound by which sulfur trioxide and N,N-dimethylformamide(DMF) are useful sulfating agents for carbohydrates. DMF is readily available and its complex with sulfur trioxide is quite stable. Also, DMF is an excellent solvent for a great number of carbohydrates.

Wheat straw, newsprint paper, kraft paper, lard, fatty alcohol, filter paper and cellulose powder were used as starting materials. Treating the starting materials with DMF prior to the sulfation apparently activates the starting material, possibly by association of the highly

polar DMF with the polar hydroxyl groups. If omitted or substituted with other solvents, such as alcohol and acetone, an incomplete reaction results, and an inferior product is obtained.

Figure 7 shows the COD removal efficiency of sulfated carbohydrate using treated and untreated kraft paper with DMF. It is obvious that the effect of pre-swelling of starting materials is quite significant. The COD removal efficiency was 83 percent for treated material, compared with 49 percent for untreated material.

Figure 8 shows the effectiveness of various kinds of swelling agents. Kraft paper was blended with DMF, ethyl alcohol, acetone and water, and soaked for two hours before reacting with DMF-SO_3 complex. All the sulfated kraft paper was refined in the same manner, and was used to precipitate protein from wastewater. The results of COD removal efficiency tests revealed that DMF is the best swelling agent tested.

Quality control during preparation of DMF-SO_3 complex is important for carbohydrate sulfation. Two batches of DMF-SO_3 complex were prepared under slightly varying reaction conditions. Significant differences in the results of precipitation were observed. The reaction of DMF and SO_3 was highly exothermic, and was carried out at a temperature below 40° C. The first batch of DMF-SO_3 complex was prepared, using 150 ml. of DMF and 90 g. of SO_3. Temperature was maintained at about 35° C. throughout the reaction and yellowish DMF-SO_3 complex was obtained. The second batch was prepared with the same weight of DMF-SO_3 as the first batch. However, at the middle of the reaction, part of the SO_3 became solidified and caused the rate of addition of SO_3 to be temporarily out of control. A burner was applied to keep SO_3 from solidifying. As a

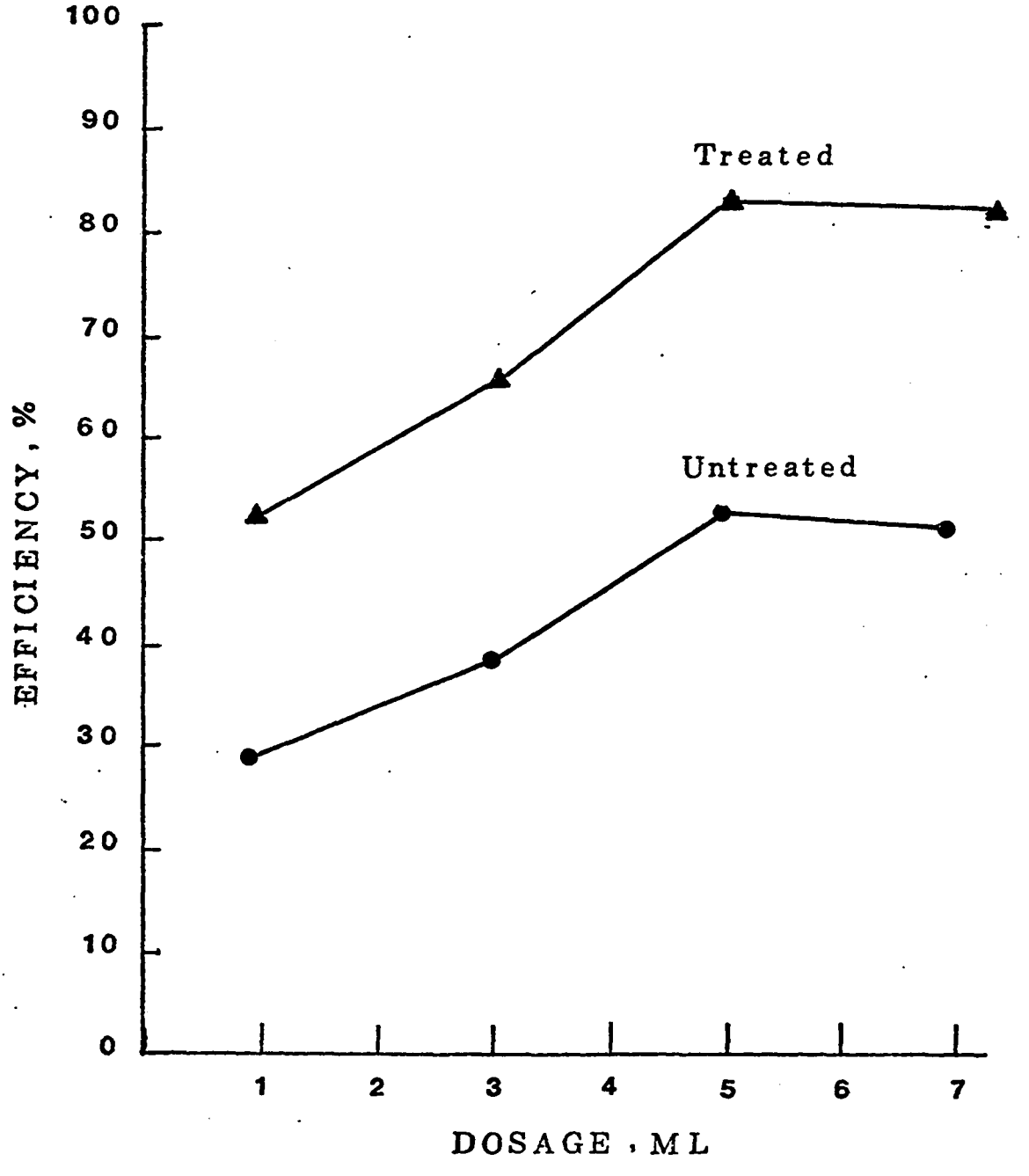


Figure 7. Comparison of COD Removal Efficiency of Sulfated Kraft Paper Prepared with Treated and Untreated Paper with DMF.

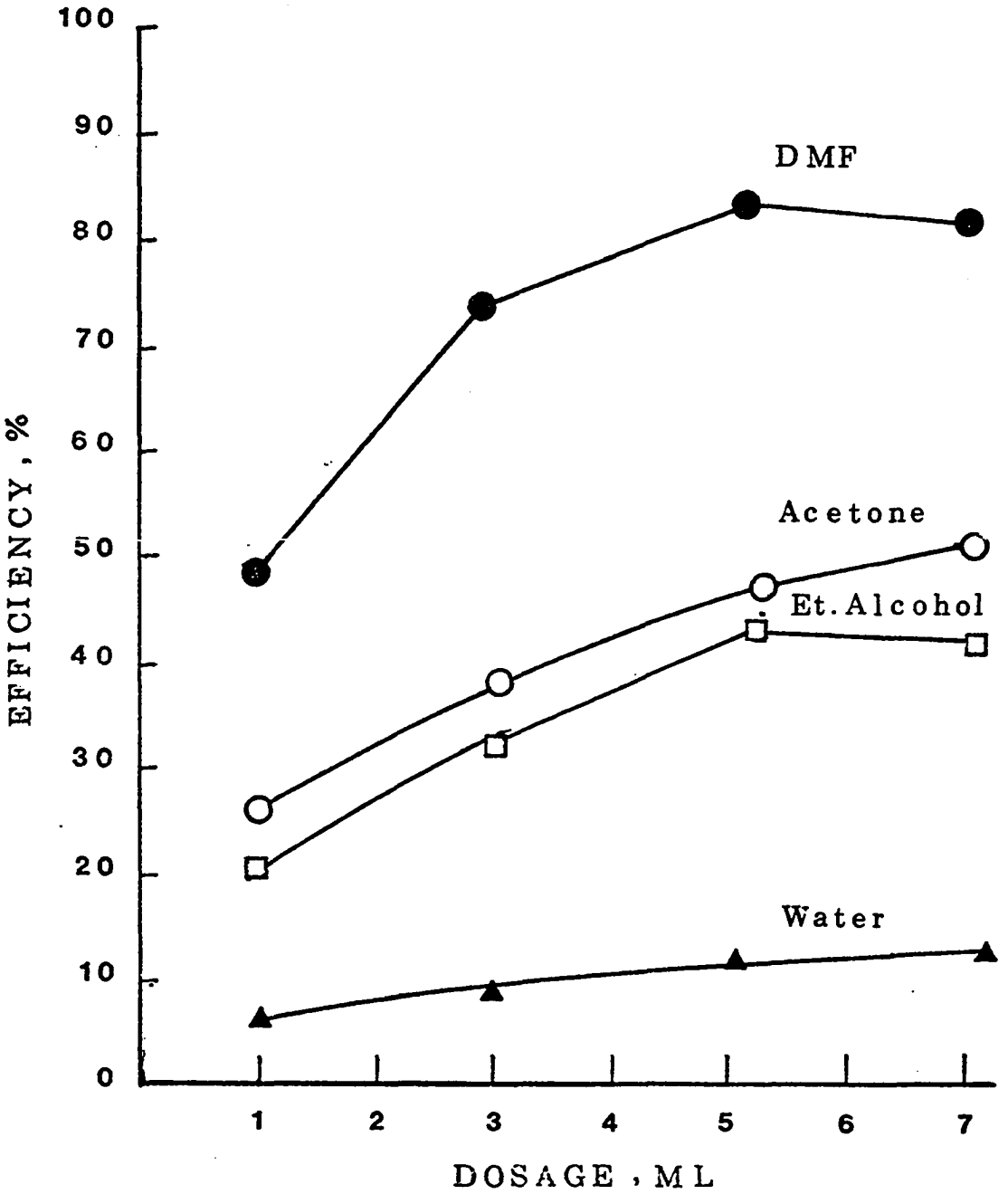


Figure 8. Comparison of COD Removal Efficiency of Sulfated Kraft Paper Prepared with Different Swelling Agents.

result, an undetermined amount of SO_3 was lost due to evaporation. A creamish DMF-SO_3 complex was obtained at the end of the reaction.

Both batches of DMF-SO_3 complex were used as sulfation agents for newsprint and kraft paper. The resulting COD removal efficiencies using the sulfated carbohydrates are given in Tables A-3, A-4 and Fig. 9. There is a significant difference in COD removal efficiency between two sulfated carbohydrates which were prepared with different batches of DMF-SO_3 complex. Eighty-three percent of COD removal efficiency was obtained using the sulfated kraft paper which was prepared with batch No. 1 DMF-SO_3 complex compared to only 55 percent using batch No. 2.

Although the presence of some excess DMF is necessary as a diluent, a large excess is disadvantageous because the rate of reaction is considerably decreased. It was determined that for 10 g. of kraft paper, an excess of 100 ml. of DMF is necessary to keep the paper fiber suspended. The excess amount of DMF must be removed from the raw product; otherwise, a significant amount of COD will be introduced to the treated effluent.

Sulfation of carbohydrates is a function of reaction temperature, time, and the ratio of sulfation agent to carbohydrates. Sulfation of carbohydrates with a DMF-SO_3 complex was performed under controlled conditions to determine the effect of temperature, reaction time, and ratio of sulfation agent ot carbohydrate.

In an experiment, 10 g. of kraft paper was pre-swollen with DMF for 2 hours prior to mixing with a DMF-SO_3 complex at 25° C. Portions of the reaction mixture were withdrawn at definite intervals of time and the sulfated kraft paper was recovered and analyzed to determine

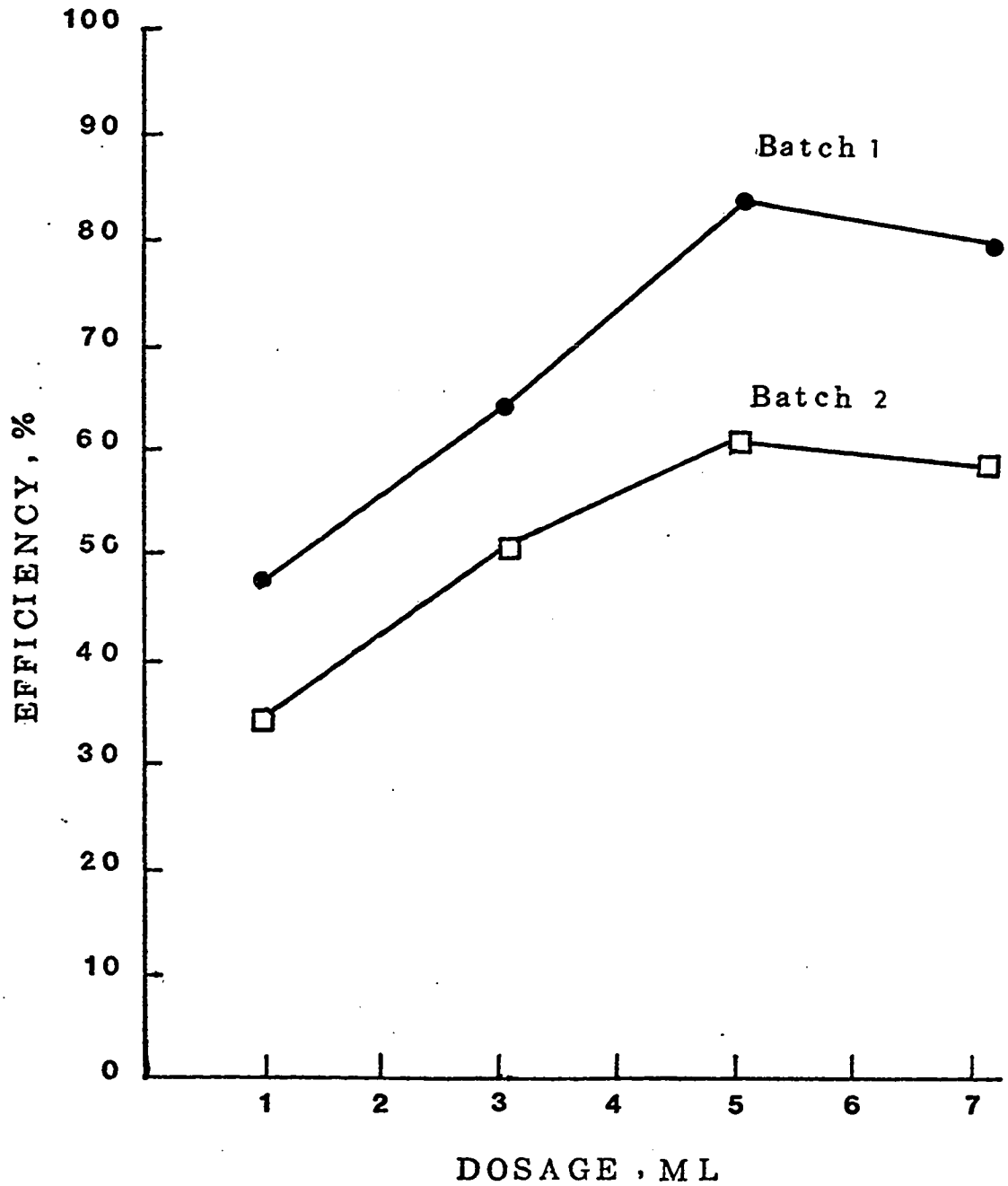


Figure 9. Effect of DMF-SO_3 Complex on GOD Removal Efficiency of Sulfated Kraft Paper.

the progress of sulfation with time. It was found that the maximum sulfation took place after one hour of mixing the reactants. Continued mixing did not increase the degree of sulfation. The degree of sulfation and COD removal efficiency of the products at different intervals of time are presented in Table A5 and Figure 10. From the study of the progress of sulfation with time, it was found that sulfation occurred very rapidly in the first 20 minutes and was virtually complete within one hour. Increasing the reaction time to beyond two hours decreased the degree of sulfation.

Table A6 and Figure 11 show the temperature dependence of the sulfation reaction. In general, a high rate would be observed at an elevated temperature. The experimental results did not follow the expected trend. It was reported that the sulfation was slow at temperatures below 0° C. The maximum sulfation took place at 35° C. and no improvement was observed at higher temperatures (Table A6). The sulfation reaction was slightly exothermic. The reason lower rates of sulfation were observed at higher temperatures was not clear; however, it may be due to the destruction of carbohydrate by the sulfation agent at higher reaction temperatures.

Sulfation was also a function of the ratio of DMF-SO_3 complex to carbohydrates. The yield of sulfated product is dependent on the amount of the sulfation agent used in the reaction. When insufficient quantities of sulfating agents were used, a lower yield of product was obtained. The amount of untreated carbohydrate increased with decreasing amounts of sulfating agent. Apparently, the reaction occurs at the surface of the carbohydrate fiber. As soon as the sulfate ions are

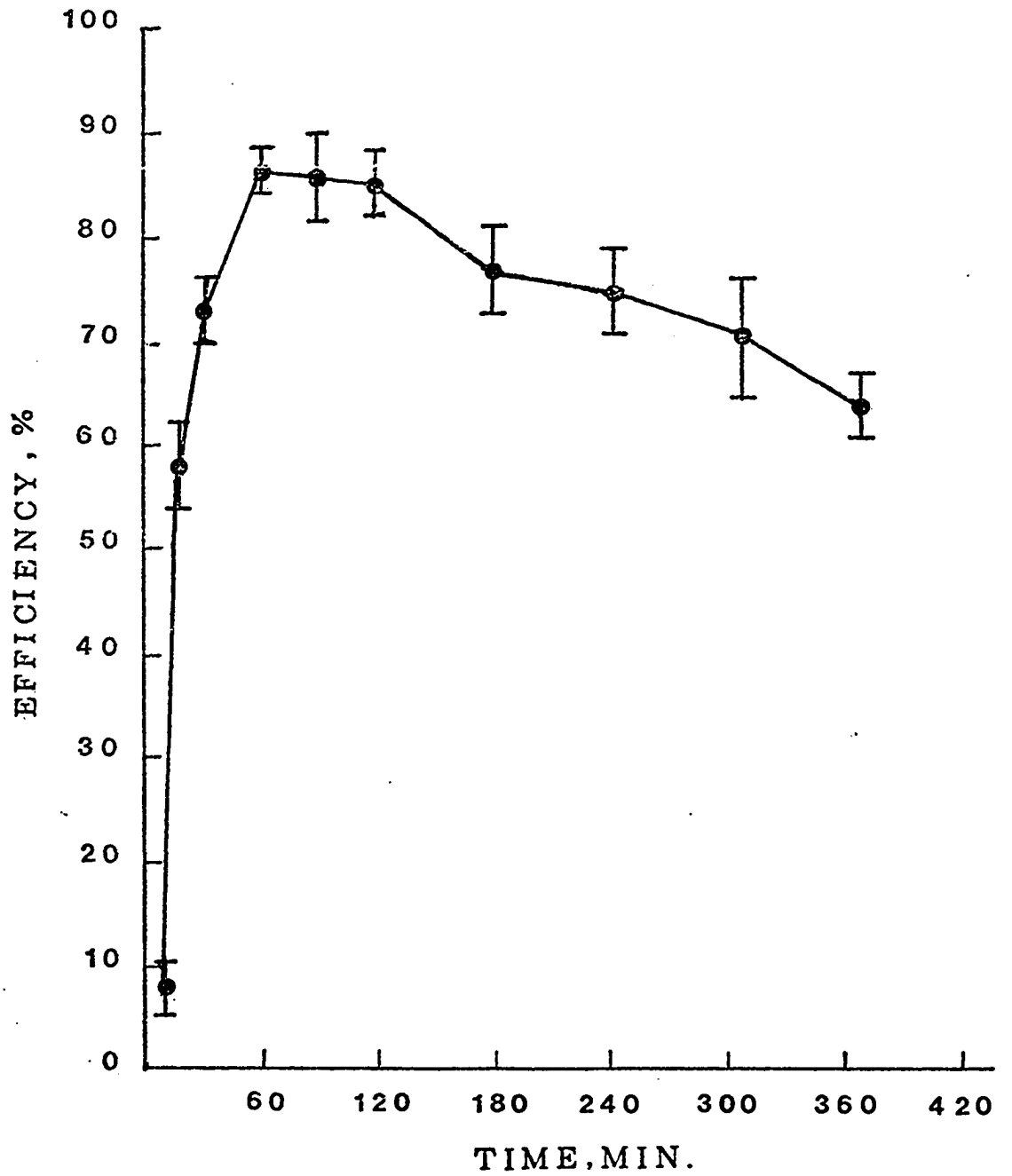


Figure 10. Effect of Reaction Time on COD Removal Efficiency.

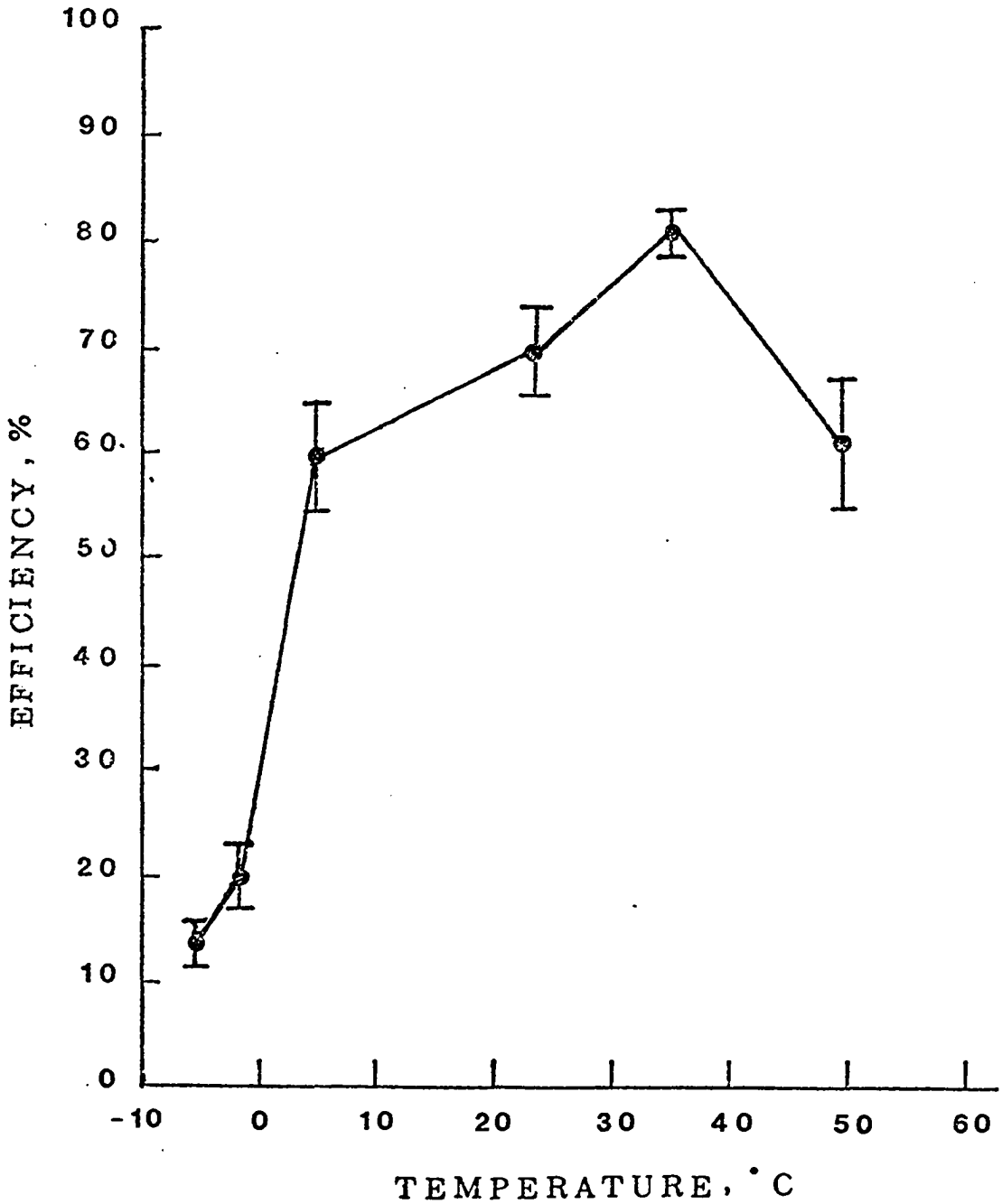


Figure 11. Effect of Temperature on Sulfation of Carbohydrate.

substituted for hydroxide groups of the fiber molecules, the surface of the fiber becomes soluble and peels off, and substitution of sulfate for hydroxide can proceed further until the macromolecules of the fiber become sulfated to the maximum possible extent. This so-called peeling process probably applies mainly to the highly crystalline regions of the fiber molecules. The amorphous portion presumably is penetrated quite rapidly by the reaction medium, and becomes substituted without difficulty. In a typical run, 50 g. of DMF-SO_3 complex was used for sulfating 10 g. of kraft paper. When the reaction was completed, the kraft paper was almost dissolved, resulting in a viscous product. Trace amounts of unreacted paper fiber could be seen in the reaction product. In several trials, 30 g. of DMF-SO_3 complex was used for sulfating 10 g. of kraft paper. At the end of the reaction, a significant fraction of the paper was unreacted. The reaction product was separated by filtration. The filtrate was purified and used for protein precipitation; the unreacted paper fiber was air-dried and weighed. The results of sulfation by using different amounts of DMF-SO_3 are summarized in Table A7 and Figure 12.

It was found from these studies that the sulfating agent must be used in excess for satisfactory results. Previous investigations (Takiura, 1970) report that various ratios of sulfating agent to carbohydrate were used. Several investigators have used a sulfating agent to carbohydrate ratio of 2:1. Due to the molecular weight of various carbohydrates, it was not practical to determine the ratio of sulfating agent to carbohydrate on a mole to mole basis. In order to determine the optimum ratio of sulfating agent to carbohydrate, the weight-to-weight ratio from 2:1 to 8:1 was used during the course of this study. According to the literature, in

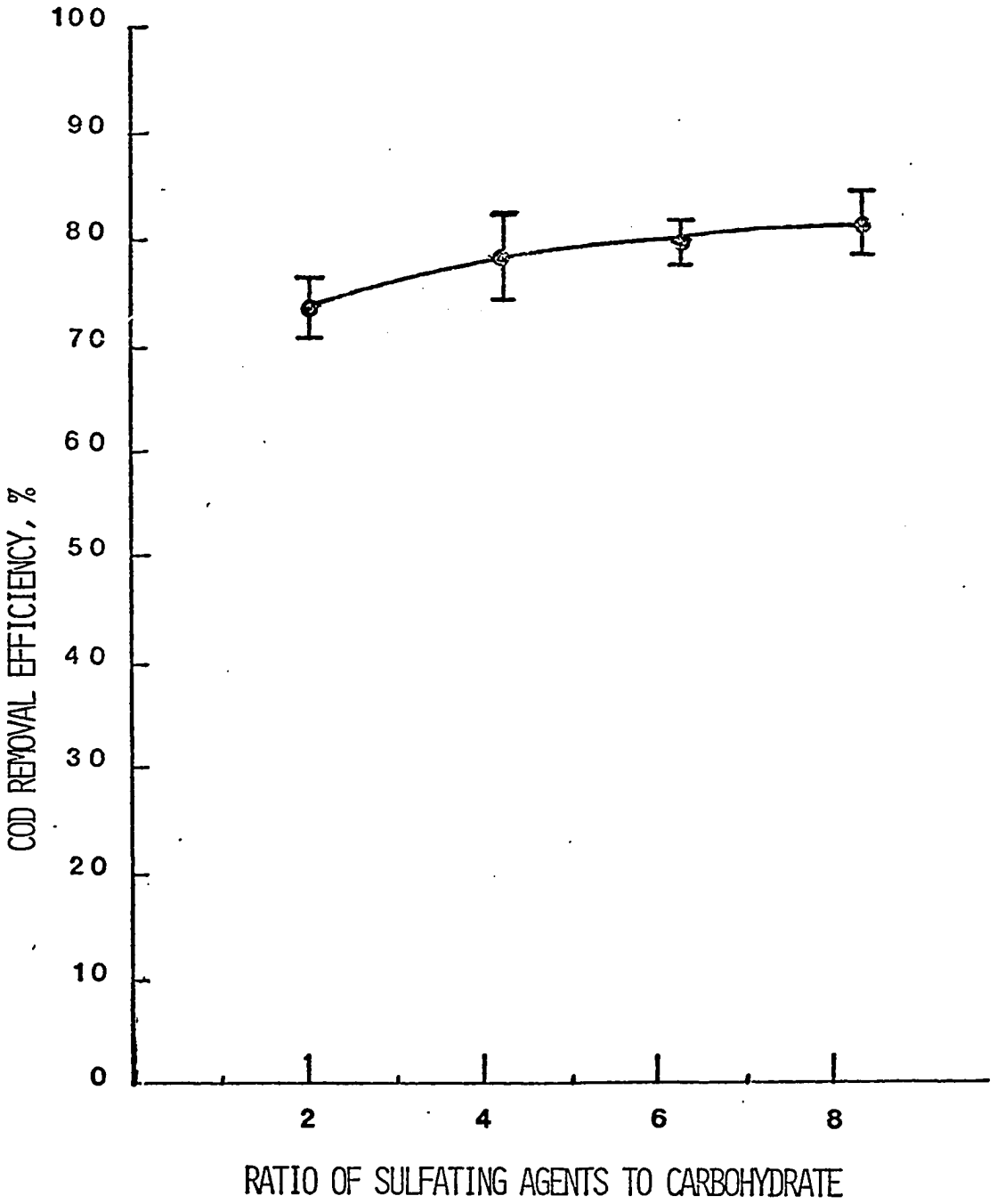


Figure 12. Relationship between Ratio of DMF-SO_3 Complex to Carbohydrate and COD Removal Efficiency.

order for sulfation to take place a concentration of at least 65 percent of concentrated sulfuric acid is necessary.

The economic aspect needs to be taken into consideration when the optimum ratio is determined. The concentrated sulfuric acid, DMF, and sulfur trioxide are the more expensive starting materials compared with kraft paper. The amounts of these materials used in the sulfation will become a determining factor in evaluating the cost effectiveness of the method. The COD removal efficiency of sulfated kraft paper when prepared with different ratios of a DMF-SO_3 complex to carbohydrate was evaluated. The results are presented in Figure 12.

It may be seen from the above figure that the COD removal efficiency increases gradually from 2:1 to 4:1. The change of COD removal efficiency between 4:1 and 8:1 was not significant. The experiment was repeated with sulfation of DMF-SO_3 complex and newsprint paper, and a similar result obtained. The peak COD removal efficiency occurred at a ratio of 4.5:1. Therefore, it is determined that the optimum ratio of DMF-SO_3 complex to either newsprint or kraft paper is about 4:1.

Previous work showed that the sulfated carbohydrate is usually stored in its diluted acid form (Thorn, 1970, Takiura, 1970), but in this form it is subject to autohydrolysis with a subsequent loss of weight and deterioration of its colloidal properties. This study shows that the sulfated carbohydrate can be converted to a sodium salt and dry acid form. Both are stable in air and can be stored for extensive periods of time. The sodium salt of sulfated carbohydrate can be used as a protein precipitating agent. It was also found that by using a DMF-SO_3 complex as a sulfating agent and DMF as reaction medium, the sulfated carbohydrate

can be recovered in its dried acid form.

The yields of sodium carbohydrate sulfate after neutralization with sodium hydroxide are quantitative with respect to the starting materials. Table 7 shows that the kraft paper yields more sulfated carbohydrate than other starting materials. It may also be seen from Table 7 that with 10 g. of carbohydrate and 50 g. of a DMF-SO_3 complex, only an average of 4 g. of sulfated carbohydrate results if the product is a sodium salt, compared to average of 19 g. of sulfated carbohydrate if the product is a dry acid form.

Both the sodium salt and acid form of sulfated carbohydrate were used as a protein precipitating agent to remove protein from wastewater. A larger quantity of acid form of sulfated carbohydrate was obtained, however, more concentrated solution was necessary to achieve comparable COD removal efficiencies than when a sodium salt of sulfated carbohydrate was used. The result of COD removal efficiency of both chemicals for beefwaste and egg-white solution is given in Table A8. The results show that using 3 ml. of a 0.5 percent solution of the sodium salt or 1.0 percent of the acid form of sulfated kraft paper, newsprint paper and filter paper comparable COD removal efficiencies were achieved.

A DMF free, dry acid form of sulfated carbohydrate was obtained by washing the raw product of sulfated carbohydrate with ethyl alcohol. The washing mixture contains ethyl alcohol, DMF and trace amounts of water. These three components have a wide range of boiling points. Their boiling points are 68, 100 and 153° C. for ethyl alcohol, water and DMF, respectively. They can be separated easily by conventional distillation methods. The mixture from the washing process may be collected after each

Table 7. Comparison of Product Yield as Sodium Salt and Acid Form

Experiment	Carbohydrate	Starting Material		Product		Yield
		$DMF-SO_3$ Complex	DMF	Sodium Salt	Acid Form	
		g	g	g		%
A - 28	Kraft Paper, 10g	50	94	—	21.9	14.2
A - 23	Newsprint Paper, 10g	50	94	—	18.8	12.2
A - 24	Filter Paper Paper, 10g	50	94	—	15.4	10.0
A - 7	Kraft Paper, 10g	50	94	4.5	—	2.9
A - 6	Newsprint Paper 10g	50	94	4.2	—	2.7
A - 4	Filter Paper 10g	50	94	3.9	—	2.5

washing and composited for distillation. The distillate collected between 75 to 90° C. was reused in subsequent washing processes. This portion of the distillate was recycled four times to determine the effect on the protein removal efficiency. The volume of distillate collected at different temperature ranges during four reuse cycles is presented in Table 8. The COD removal efficiency of the sulfated carbohydrate when prepared with recycled alcohol is given in Table 9 and Figure 13. The results show no significant difference when recycled alcohol is used in place of unrecycled alcohol.

Based on the volume of the alcohol collected at 75-90° C., approximately 80 percent of the alcohol is reusable. The distillate collected at different temperature ranges was analyzed qualitatively using gas chromotography. By comparing the spectra of the portions of distillate with the spectra of analytical grade of ethyl alcohol and DMF, it is shown that alcohol and trace amounts of water were collected at the temperature range 75-90° C.; a mixture of DMF, alcohol and water was collected between 90 and 135° C.; and the portion collected beyond 135° C. was DMF. Based on the volume collected, it was estimated that 75 percent of DMF could be reused.

Reuse of alcohol and DMF mixture which was collected at 90-135° C. as a solvent or reaction media was not successful. This is probably due to the insufficient amount of DMF present in the mixture.

The mechanism of the reaction between the sulfated carbohydrate and the proteins is not well understood. The reaction appears to be one of cross-linking, presumably by formation of a salt between the negative sulfate groups of sulfated carbohydrate and the basic sites of the

Table 8. Distillation of Ethyl Alcohol and DMF Mixture

Temperature Range °C	Volume Collected			
	1st Cycle	2nd Cycle	3rd Cycle	4th Cycle
75 - 90	305	291	280	260
90 - 135	62	39	44	53
135 -	2	4	5	4

Table 9. Effect of Recycled Alcohol on COD Removal Efficiency

Waste	Dosage ml	COD Removal Efficiency, % 1st Cycle	2nd Cycle	3rd Cycle	4th Cycle
Beef waste	3	79	81	79	71
	5	81	90	82	80
	7	83	90	85	86
Egg White Solution	3	56	53	53	55
	5	58	55	55	54
	7	57	59	57	57

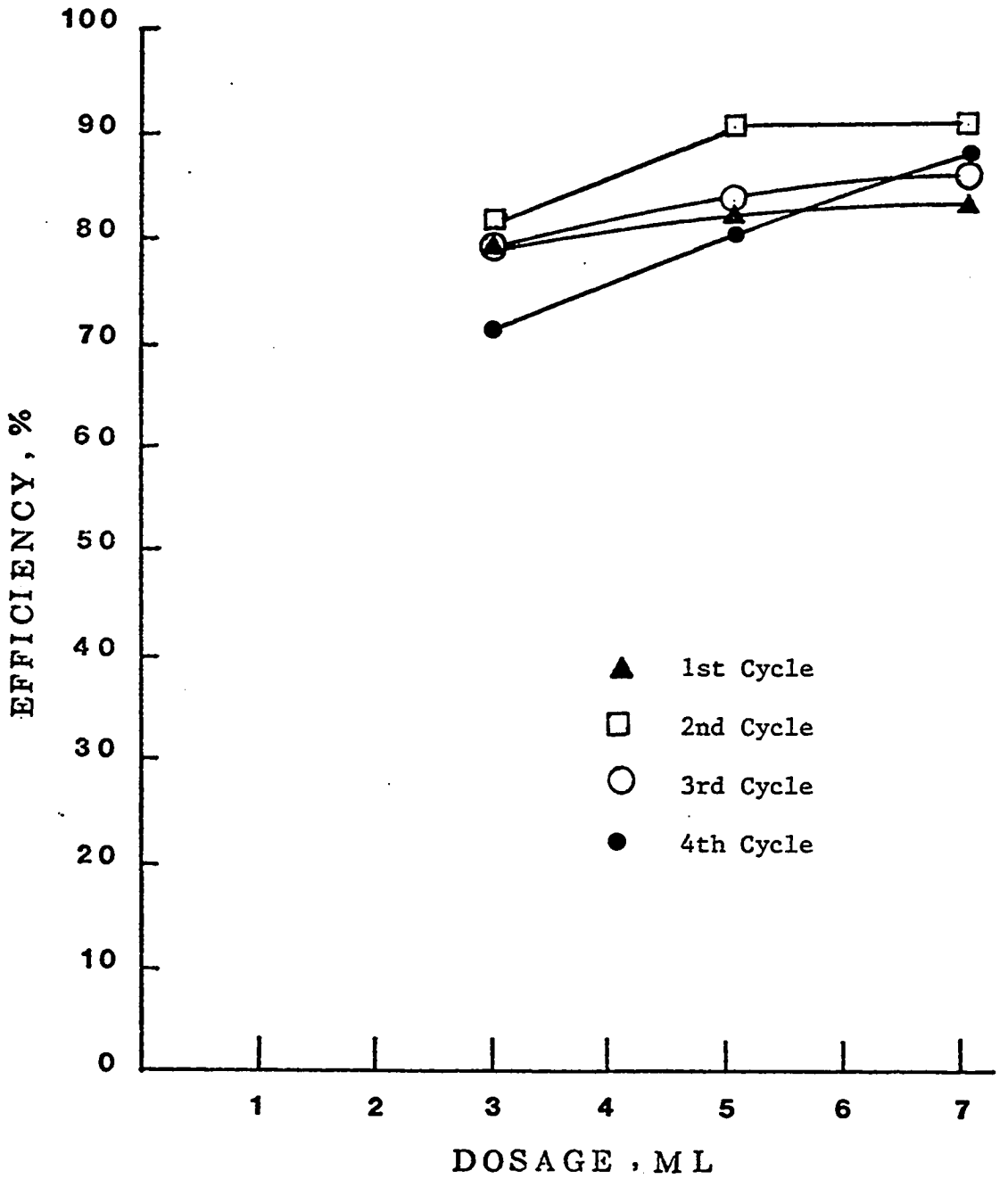


Figure 13. Effect of Recycled Alcohol on COD Removal Efficiency of Beef Processing Wastewater.

protein. The functional sulfate group of the sulfated carbohydrate was identified with an infrared spectrophotometer. A strong sulfate peak was observed in the spectrum at about 3400 cm^{-1}. This value definitely originates from the bonded hydroxyl groups, since unmodified carbohydrate exhibits intensive, intermolecular hydrogen-bonding, which makes the carbohydrate insoluble in water. The infrared spectra of sodium carbohydrate sulfate are given in Figs. A1 and A2. The spectrum of the acid form of carbohydrate sulfate is given in Figs. A3 and A4.

Evaluation of Products

The sulfated kraft paper prepared with a DMF-SO_3 complex under optimum reaction conditions was used as a precipitation agent to remove protein from meat-packing processing wastewater, egg-white solution and simulated beef processing wastewater (SBP). The optimum experimental conditions for bench scale tests were determined. The protein removal efficiency of sulfated kraft paper was compared with other commercially available sulfated marine algae products, lignosulfonic acids and corn syrup sulfates.

A one percent solution of sulfated kraft paper was used to treat the meat-packing wastewater, egg-white solution, and SBP wastewater. Results of the test are given in Table A14. The relationship between the chemical dosages and efficiency of COD removal is shown in Fig. 14. The results show that the best floc formation with an optimum dosage of six ml. (0.004 g/l) for each five hundred ml. of waste sample gave reduction of COD, TSS, Org-N, and O & G of 84, 98, 65 and 94

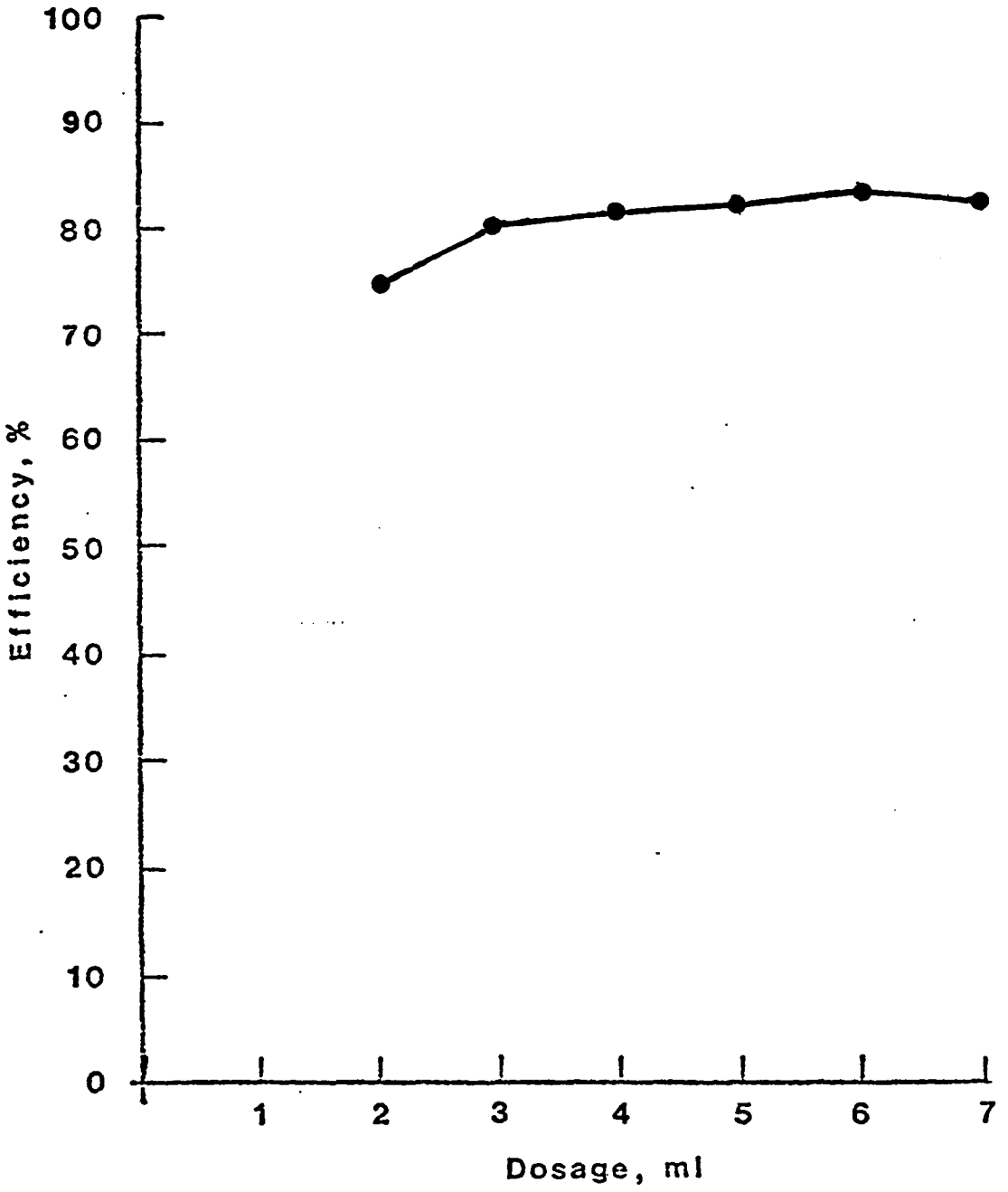


Figure 14. Efficiency in the Removal of COD by Addition of Different Amounts of Kraft Paper Sulfate.

percent, respectively.

Similar tests were performed for lignosulfonic acid (LSA). A 15 percent LSA solution was used for protein precipitation. Jar tests were run to determine the influence of pH on LSA protein precipitation. Results of the influence of pH are shown in Table 10.

After the pH range was determined, the next step was to evaluate the optimal ratio between LSA and protein for most effective removal. The result was shown in Fig. 15. Experimental evidence shown in Table A15 confirm that the efficiency of treatment of proteinaceous waste with LSA is dependent upon the LSA dosage. Results from the chemical analysis of the effluent show optimal reductions of COD, TSS, Org-N, and O & G of 82, 99, 70 and 95 percent, respectively.

Bench scale studies were conducted to evaluate the effectiveness of Viscarin 402 Carrageenan on protein precipitation. Preliminary jar tests were run to determine the suitable pH range. Table 11 summarizes the characteristics of precipitation of carrageenan-protein at various pH ranges. The results indicate that the pH range of 2.8 to 3.1 is the optimum to achieve effective precipitation. A bench scale study was used to determine the relationship between the organic removal efficiency and chemical dosages. The results are presented in Fig. 16. The supernatent of the treated samples were analyzed, and results of the tests are shown in Table A16. Optimal organic removal was obtained at a pH of approximately 3, with the optimum dosage of sixteen ml. (0.32 g/l). This gave COD, TSS, Org-N, O & G reductions of 79, 80, 51 and 81 percent, respectively. The supernatant obtained from chemical precipitation was not clear. The floc was large and

Table 10. Effect on pH on LSA-Protein Precipitation

pH Range	Precipitation
0 - 1	Poor
2 - 3	Good
3.4 - 4.5	Poor
Above 4.5	None

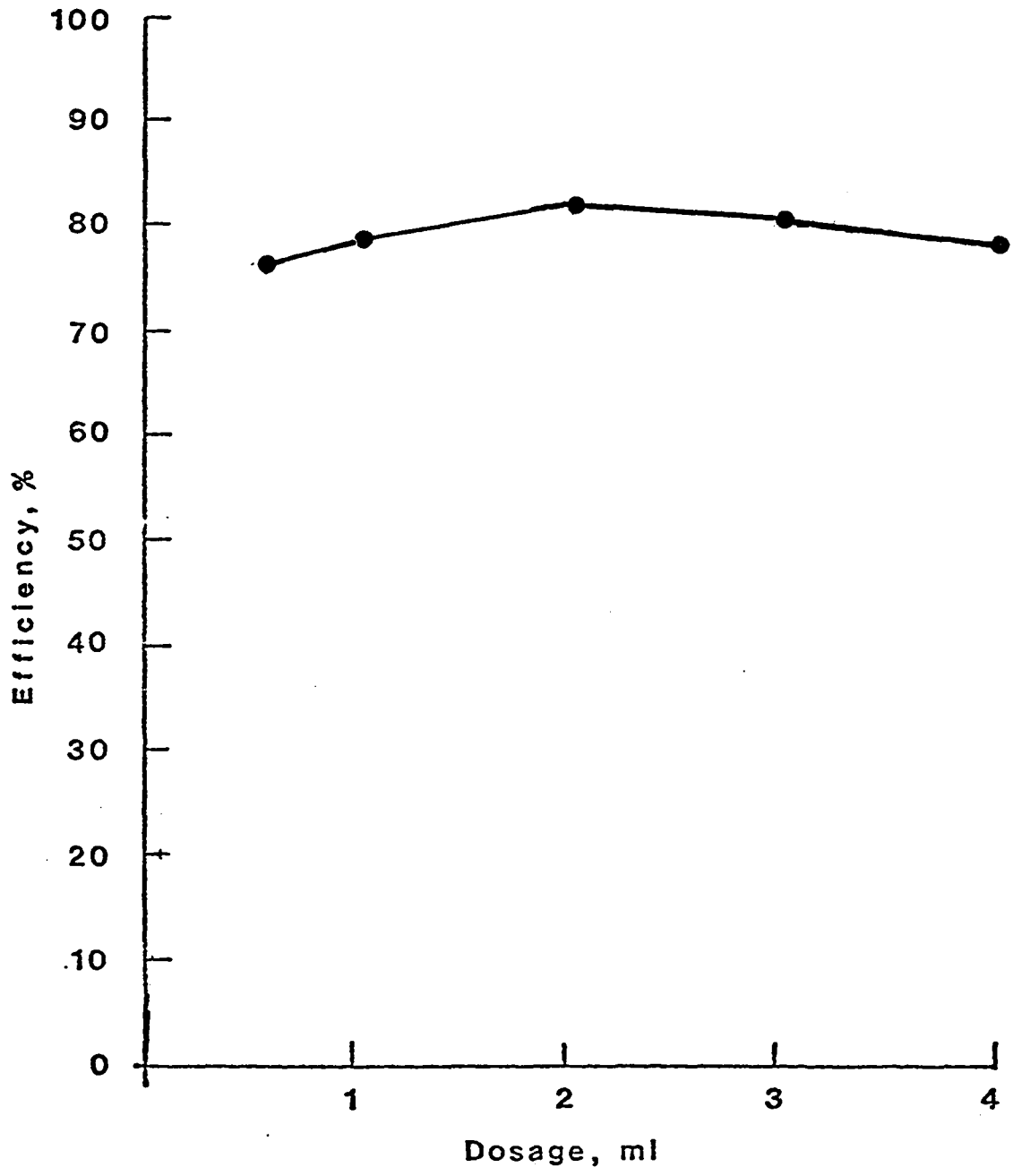


Figure 15. Efficiency in the Removal of COD by Addition of Different Amounts of Lignosulfonic Acid.

Table 11. Effect of pH on Carrageenan-Protein Precipitation

pH Range	Precipitation
0 - 1	Poor
2 - 2.7	Fair
2.8 - 3.1	Good
Above 3.5	Poor

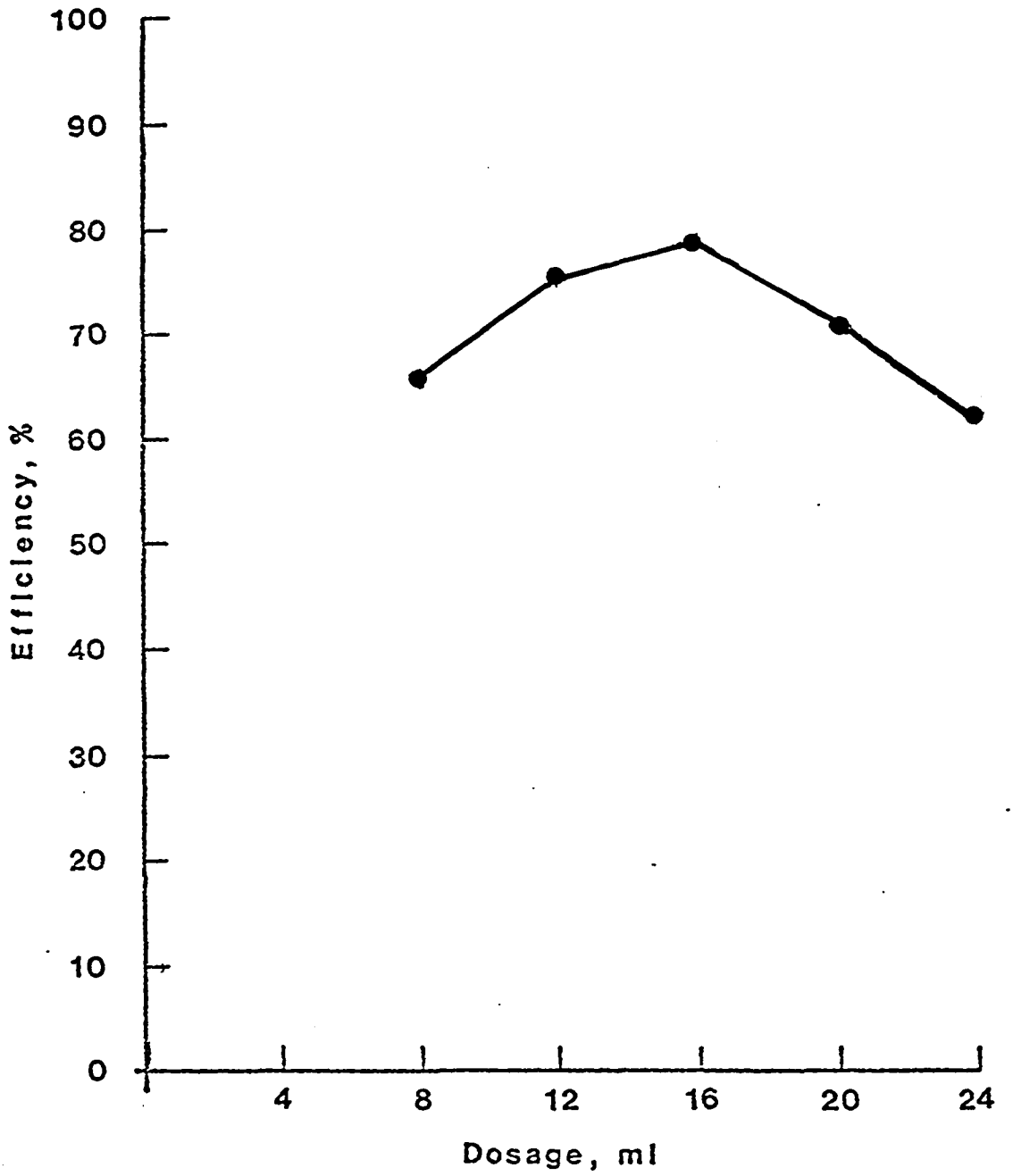


Figure 16. Efficiency in the Removal of COD by Addition of Different Amounts of Viscarin 402 Carrageenan.

fragile, with a tendency to float. Gentle stirring would break up the floc, creating a cloudy effluent.

A comparison of COD removal efficiency using kraft paper sulfate, corn syrup sulfate, lignosulfonic acid, and Viscarin 402 Carrageenan as precipitating agents is based on the optimal dosage of precipitating agents to treat 500 ml of beef waste. Table 12 indicates that approximately 80 percent COD removal efficiency is achieved using the optimal dosage for each of the precipitating agents. It requires 0.06, 0.10, 0.45, and 0.16 g of kraft paper sulfate, corn syrup sulfate, lignosulfonic acid, and Viscarin 402 Carrageenan, respectively, to achieve this removal efficiency. Based on the amount of material used, kraft paper sulfate is the most effective precipitating agent.

Cost Analysis

The economic analysis is based on the cost of the starting materials to prepare a sulfated carbohydrate with the optimum dosage for COD removal. Capital, operational and maintenance costs are not included in the analysis.

The itemized material costs of the various methods of preparation are given in Table 13. Calculations are based on the treatment of one million gallons of beef-processing wastewater. The total cost for treatment, using sulfated corn syrup and sulfated kraft paper is $72 and $350, respectively. A considerable amount of DMF is used in the preparation of sulfated kraft paper. The cost is reduced from $350 to $84, when 75 percent of the DMF was reused.

The experimental results show that COD removal efficiency of

Table 12. Evaluation of Precipitating Agents

Precipitating Agent	Concentration %	Optimal Dosage ml	Optimal Dosage g	C O D Removal Efficiency %
Kraft Paper Sulfate	1.0	6.0	0.06	84
Corn Syrup Sulfate	20	0.5	0.10	80
Lignosulfonic Acid	15	3.0	0.45	82
Viscarrin 402 Carrageenan	1	16	0.16	79

* Based on the treatment of 500 ml of beef wastewater with COD of 5,000 mg/L.

Table 13. Cost Analysis of Starting Materials.

Sulfated Carbohydrate	Starting Materials	Optimum* Dosage g/L	Price $/ton	Cost for Treatment $/Mgal Materials	Total
Sulfated Corn Syrup	Corn Syrup	0.07	159.40	46.56	71.97
	H_2SO_4	0.13	46.85	25.41	
Sulfated Kraft Paper (Acid Form)	Paper	0.02	5.00	0.42	355.39
	D M F	0.09	930.00	349.03	
	SO_3	0.01	142.50	5.94	
Sulfated Kraft Paper (Acid Form with DMF Reused)	Paper	0.02	5.00	0.42	83.92
	D M F	0.02	930.00	77.56	
	SO_3	0.01	142.50	5.94	

* Reslts are based on treatment of beef processing wastewater with approximate COD 5,000 mg/L.

0.5 ml of 20 percent of sulfated corn syrup and 3 ml of one percent of sulfated kraft paper are comparable. However, the total cost of sulfated corn syrup is lower than the total cost of sulfated kraft paper. It appears that DMF is the cost-determining factor. More DMF can be reused when the process is expanded to an industrial scale. The cost of sulfated kraft paper will be comparable with the cost of sulfated corn syrup. Based on the results of the economic analysis in this study, corn syrup and concentrated sulfuric acid are the choice for starting materials.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The objective of this study is to develop a consistent and economically feasible method to prepare sulfated carbohydrate for treatment of high protein wastewater. Several raw materials were selected for sulfation with concentrated sulfuric acid and a DMF-SO_3 complex. The optimal experimental conditions were determined. The sulfated carbohydrate was used as a protein precipitation agent for high protein wastewater. The protein removal efficiency was evaluated by the determination of COD of wastewater before and after chemical treatment. The optimal dosage for protein precipitation was also determined. Based on the treatment of waste from a meat (beef and pork) processing facility with a COD of 5,000 mg/L, the chemical cost of preparation of sulfated carbohydrate was evaluated.

In conclusion, the results of this study show that:

1. The sulfated carbohydrate can be prepared using concentrated sulfuric acid or a DMF-SO_3 complex as a sulfating agent. Raw materials, such as corn syrup, newsprint or kraft paper were used as a source of carbohydrate.

2. Concentrated sulfuric acid is an inexpensive and stable sulfating agent. It is easy to use, relatively safe to handle, and does not require equipment for storage. However, concentrated sulfuric acid is a mild sulfating agent, so it can only be used for sulfation of simple sugars or starch.

3. DMF is an excellent solvent for a number of carbohydrates. A high degree of sulfation can be achieved by swelling the carbohydrate for at least two hours prior to sulfation.

4. A DMF-SO_3 complex is a stable and excellent sulfating agent for preparing sulfated carbohydrates. Control of the reaction temperature is important in the preparation of this complex.

5. The optimum experimental conditions for sulfation of newsprint paper and draft paper using a DMF-SO_3 complex as a sulfating agent are:

(1) Reaction temperature: 35° C.

(2) Reaction time: one hour.

(3) Ratio of sulfating agent to carbohydrate (weight-to-weight): 3.5 : 1.0.

6. The sulfated carbohydrate can be refined as a sodium salt, or in a dry acid form. The sodium salt and dry acid forms are both stable in air and water soluble. A higher yield of the acid form of sulfated carbohydrate was obtained than of the sodium salt; however, a more concentrated solution of the acid form was necessary to achieve comparable COD removal efficiencies than when a sodium salt was used.

7. The refined sulfated carbohydrate can be used as a protein precipitating agent in high protein wastewater. Average removal

efficiencies of 80, 97, 94, and 65 percent were achieved for COD, TSS, O&G, and Org-N, respectively. Following the precipitation, the sludge can be separated by sedimentation, dissolved air flotation or centrifugation. After pH adjustment, the effluent of the wastewater can be discharged directly to a biological treatment system.

8. The optimal pH for protein precipitation is about 3.5. The optimal dosage for treatment of one liter of beef process wastewater (COD, 5,000 mg/L) is 0.20 g of sulfated corn syrup, 0.12 g of sulfated kraft paper, 0.90 g of Lignosulfonic acid, and 0.32 g of Viscarrin 402 Carrageenan. Based on the amount of material used, sulfated kraft paper is the most effective precipitating agent.

9. Ethyl alcohol and DMF used in the preparation of sulfated carbohydrate can be reused. The quality of the sulfated carbohydrate is not affected by this recycling process. Approximately 80 percent of alcohol and 75 percent of DMF are reusable. More DMF can be reused when the process is expanded to an industrial scale.

10. Based on the optimal dosage, the chemical cost for treatment of one million gallons of beef wastewater with a COD of 5,000 mg/L is $72 for the sulfated corn syrup and $84 for the sulfated kraft paper. The cost of sulfated kraft paper will be decreased, when more DMF can be reused in an industrial scale production.

Recommendations

Recommendations for future study are:

1. A continuous plant process should be developed to prepare sulfated carbohydrate on a large scale. The method developed in the

bench scale study needs to be evaluated when it is expanded to an industrial scale. Therefore, a pilot plant study is necessary to resolve the operational difficulties that may be encountered in a plant scale production.

2. The reaction mechanism of protein precipitation with sulfated carbohydrate is not well understood. Research should be pursued in this area.

3. A detailed cost effectiveness analysis needs to be performed. The analysis should include the capital, materials, operation and maintenance costs to prepare the sulfated carbohydrate.

4. The protein precipitation treatment process should be evaluated against other alternatives for treatment of high protein wastewater. Recovery of the precipitated protein sludge as an animal feed by-product is one of the advantages of this treatment process. Evaluation of the biological value of the sludge is essential. Study is needed to determine the physiological effects of the recovered sludge on animals.

5. The sulfated carbohydrate has not successfully precipitated protein from the wastewater which contains a high percentage of blood. Development of new precipitating agents is needed to remove blood from the wastewater. A study is also needed to evaluate alternatives for treatment of blood or the separation of blood from the wastestream.

6. Sulfated carbohydrate was used as a protein precipitating agent for treatment of beef and pork processing wastewater in this study. The effectiveness of sulfated carbohydrate for treatment of other high protein wastewaters, such as dairy, poultry, and seafood, needs to be evaluated.

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APPENDICES

Table A1. COD Removal Efficiency of Treated & Untreated Carbohydrate.

	Dosage ml	COD Before Treatment mg/L	COD After Treatment mg/L	Removal Efficiency %
Treated Carbohydrate	1	4396	2110	52
	3	4396	1538	65
	5	4396	747	83
	7	4396	879	80
Untreated Carbohydrate	1	4396	3121	29
	3	4396	2725	38
	5	4396	2242	49
	7	4396	2330	47

*Dosage of 1% of Sulfated kraft paper was used.

Table A2. COD Removal Efficiency of Sulfated Carbohydrate Prepared with Different Swelling Agents.

Swelling Agent	Dosage ml	COD Before Treatment mg/L	COD After Treatment mg/L	COD Removal Efficiency
DMF	1	4766	2430	49
	3	4766	1287	73
	5	4766	905	81
	7	4766	1001	79
Acetone	1	4766	3527	26
	3	4766	3098	35
	5	4766	2764	42
	7	4766	2097	46
Ethyl Alcohol	1	4766	3813	20
	3	4766	3384	29
	5	4766	2860	40
	7	4766	2907	39
Water	1	4766	4480	6
	3	4766	4432	7
	5	4766	4289	10
	7	4766	4164	13

Table A3. Effect of DMF-SO_3 Complex on COD Removal Efficiency of Sulfated kraft paper.

Dosage ml	COD Before Treatment mg/L Batch 1	COD Before Treatment mg/L Batch 2	COD After Treatment mg/L Batch 1	COD After Treatment mg/L Batch 2	COD Removal Efficiency % Batch 1	COD Removal Efficiency % Batch 2
1	6545	6545	3403	4319	48	34
3	6545	6545	2487	3272	62	50
5	6545	6545	1113	2552	83	61
7	6545	6545	1374	2749	79	58

Table A4. Effect of DMF-SO_3 Complex on COD Removal Efficiency of Sulfated Newsprint Paper.

Dosage	COD Before Treatment mg/L Batch		COD After Treatment mg/L Batch		COD Removal Efficiency % Batch	
	1	2	1	2	1	2
1	5975	5975	3644	4063	39	32
3	5975	5975	2390	3167	60	47
5	5975	5975	1135	2509	81	58
7	5975	5975	836	2008	86	64

Table A5. Effect of Reaction Time on Sulfation of Carbohydrate.

Reaction Time min.	COD Before Treatment mg/L	COD After Treatment mg/L	Degree of Sulfation	COD Removal Efficiency %
10	4343	3995	0.0	8
20	4343	1867	1.25	57
30	4343	1172	1.61	73
60	4343	608	2.02	86
90	4343	651	1.94	85
120	4343	781	1.97	82
180	4343	1042	1.83	76
240	4343	1085	1.61	75
300	4343	1259	1.63	71
360	4343	1476	1.53	66

*Dosage of 5.0 ml of 1% of Sulfated Kraft Paper was used in COD Removal Efficiency Tests.

Results of COD removal efficiency are based on the average of 5 runs.

Table A6. Effect of Temperature on Sulfation of Carbohydrate.

Reaction Temperature °C	COD Before Treatment mg/L	COD After Treatment mg/L	Degree of Sulfation	COD Removal Efficiency %
-5	4358	3691	0.40	15
0	4358	3464	0.45	20
7	4358	2104	1.03	59
25	4358	1381	1.55	68
35	4358	854	1.92	80
50	4358	1630	1.50	62

*Dosage of 5 ml of 1% of Sulfated Kraft Paper was used in COD Removal Efficiency Tests.

Results of COD removal efficiency are based on the average of 4 runs.

Table A7. Effect of Ratio of Sulfating Agent to Carbohydrate on Sulfation.

Ratio of Sulfating Agent to Carbohydrate	COD Before Treatment mg/L	COD After Treatment mg/L	Degree of Sulfation	COD Removal Efficiency %
2	4400	1232	1.48	72
4	4400	968	1.94	78
6	4400	880	1.86	80
8	4400	336	1.90	81

*Dosage of 5 ml of 1% of Sulfated Kraft Paper was used in COD Removal Efficiency Tests.

Results of COD removal efficiency are based on the average of 5 runs.

Table A8. Comparison of COD Removal Efficiency of Sodium Salt and Acid Form of Sulfated Carbohydrate.

	Sample	Dosage ml	pH	COD Removal Efficiency Beefwaste, %	COD Removal Efficiency Egg White Solution, %
Sodium Salt (0.5% Solution)	Kraft Paper	3	3.5	82	69
	Newsprint paper	3	3.5	79	72
	Filter paper	3	3.5	72	65
Acid Form (1.0% Solution)	Kraft Paper	3	3.5	83	74
	Newsprint paper	3	3.5	80	71
	Filter paper	3	3.5	76	68

Table A9. Effect of Recycled Alcohol on COD Removal Efficiency of Beef Waste

Cycle	Dosage ml	pH	COD Before Treatment mg/L	COD After Treatment mg/L	COD Removal Efficiency, %
1st	3	3.2	4358	915	79
	5	3.2	4358	828	81
	7	3.2	4358	740	83
2nd	3	3.2	4358	828	81
	5	3.2	4358	436	90
	7	3.2	4358	436	90
3rd	3	3.2	4358	915	79
	5	3.2	4358	514	82
	7	3.2	4358	654	85
4th	3	3.2	4358	1263	71
	5	3.2	4358	872	80
	7	3.2	4358	610	86

Table A10. Results of Chemical Analyses of 20% Glucose Trisulfate.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
0.3	5.5	2,130	--	-	--	59	-	-	-
0.5	4.8	956	44	47	277	82	97	66	89
1.0	3.8	1,211	--	-	--	77	-	-	-
1.5	2.8	1,301	--	-	--	75	-	-	-
2.0	2.1	1,599	--	-	--	69	-	-	-
3.0	1.7	2,045	--	-	--	61	-	-	-
Raw	6.9	5,312	2,100	140	2,170	-	-	-	-

Table A11. Results of Chemical Analyses of 20% Corn Syrup Sulfate.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
0.3	5.5	2,125	--	74	--	60	-	47	-
0.5	4.8	1,077	60	48	130	80	97	65	94
1.0	3.9	1,156	84	55	--	78	96	60	-
1.5	2.9	1,343	115	58	--	74	94	58	-
2.0	2.0	1,487	--	-	--	72	-	-	-
3.0	1.7	2,125	--	-	--	60	-	-	-
Raw	6.9	5,312	2,100	140	2,170	-	-	-	-

Table A12. Results of Chemical Analyses of 20% Molasses Sulfate.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
0.3	5.5	2,125	--	70	--	60	-	50	-
0.5	4.9	1,296	123	48	238	76	94	65	89
1.0	3.8	1,479	--	57	--	72	93	59	-
1.5	2.9	1,625	--	60	--	69	91	57	-
2.0	2.1	1,854	--	--	--	65	-	-	-
3.0	1.7	2,340	--	--	--	55	-	-	-
Raw	6.9	5,312	2,100	140	2,170	-	-	-	-

Table A13. Results of Chemical Analyses of 20% Starch Sulfate.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
0.3	5.8	3,028	--	--	--	43	-	-	-
0.5	4.9	1,652	606	75	667	69	71	46	69
1.0	3.9	1,854	--	--	--	65	-	-	-
1.5	2.8	2,127	--	--	--	59	-	-	-
2.0	2.0	2,650	--	--	--	50	-	-	-
3.0	1.7	3,158	--	--	--	40	-	-	-
Raw	6.9	5,312	2,100	140	2,170	--	-	-	-

Figure A14. Results of Chemical Analyses of 1% Kraft Paper Sulfate.

Dosage ml	pH	Concnetration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
2	3.3	1,328	--	--	--	75	-	-	-
3	3.3	1,037	--	--	--	80	-	-	-
4	3.3	984	--	--	--	81	-	-	-
5	3.3	937	63	51	--	82	97	63	-
6	3.3	868	44	49	132	84	98	65	94
7	3.3	921	60	52	--	82	97	62	-
Raw	6.8	5,312	2,100	140	2,170	-	-	-	-

Table A15. Results of Chemical Analyses of 15% Lignosulfonic Acid.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
0.5	2.6	1,279	200	--	--	74	90	-	-
1.0	2.6	1,017	100	38	--	79	95	68	-
2.0	2.6	926	35	36	124	81	98	70	94
3.0	2.6	886	27	36	102	82	98	69	95
4.0	2.6	1,080	108	--	--	78	94	-	-
Raw	6.9	4,922	2,001	122	2,090	-	-	-	-

Table A16. Results of Chemical Analyses Of 1% Viscarin 402 Carrageenan.

Dosage ml	pH	Concentration (mg/L)				Removal Efficiency (%)			
		COD	TSS	Org-N	O&G	COD	TSS	Org-N	O&G
8	2.7	1,678	--	--	--	65	-	-	-
12	2.9	1,222	521	66	--	75	73	45	-
16	3.0	1,056	408	57	405	79	80	51	81
20	3.1	1,433	558	71	--	70	72	41	-
24	3.2	1,858	--	--	--	62	-	-	-
Raw	6.9	4,922	2,001	122	2,090	-	-	-	-

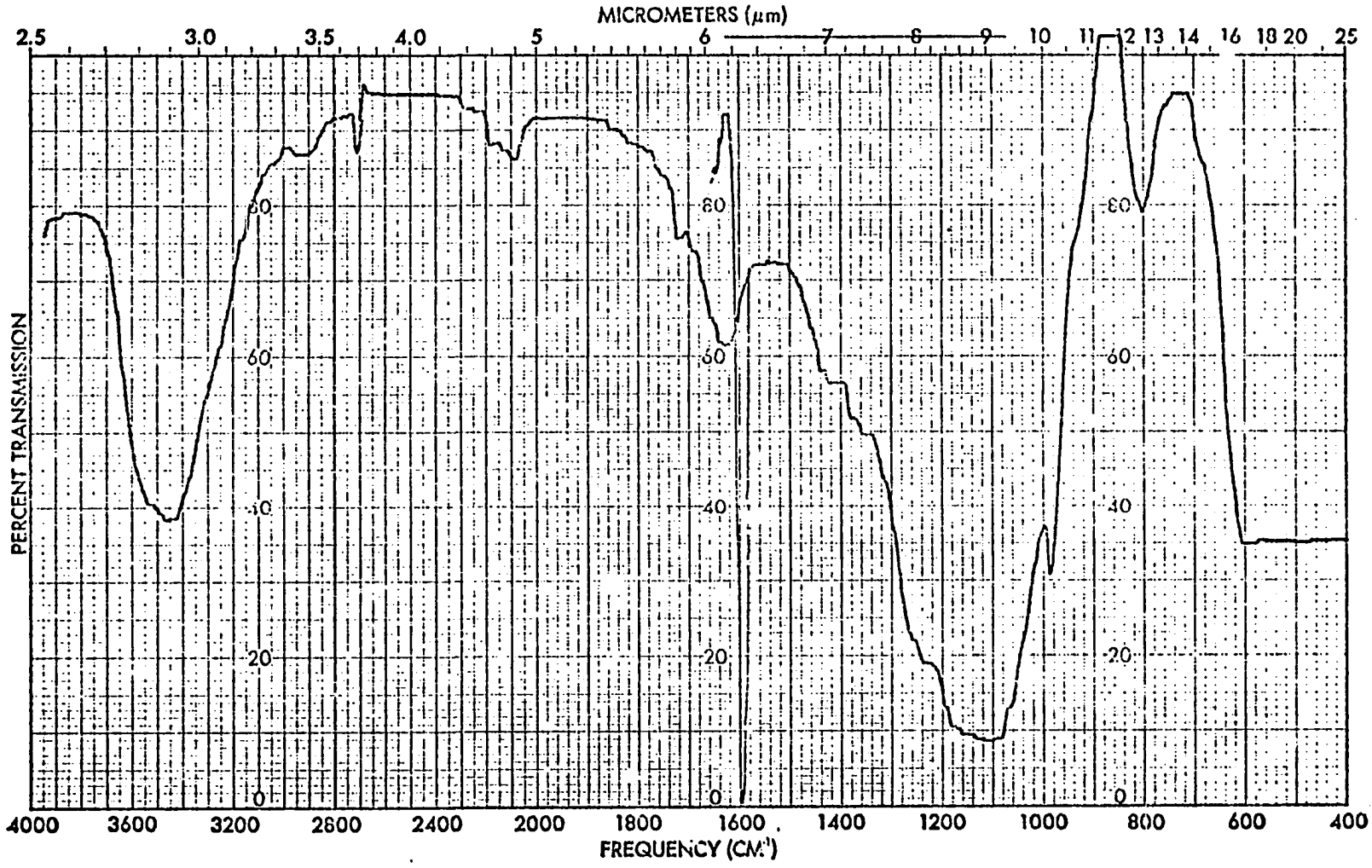

Figure A1. Infrared Spectra of Sulfated Kraft Paper (Sodium Salt).

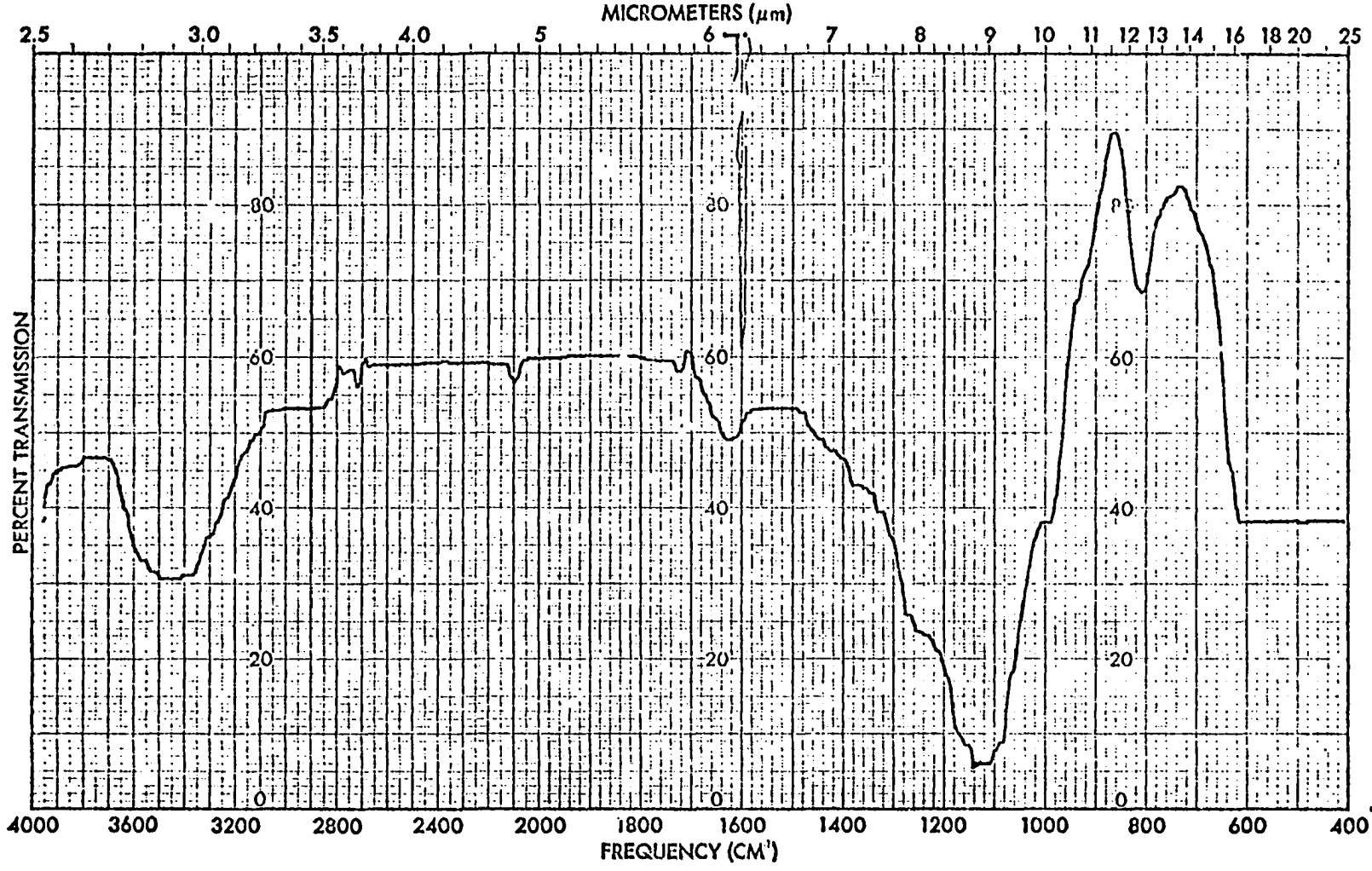

Figure A2. Infrared Spectra of Sulfated Newsprint Paper (Sodium Salt).

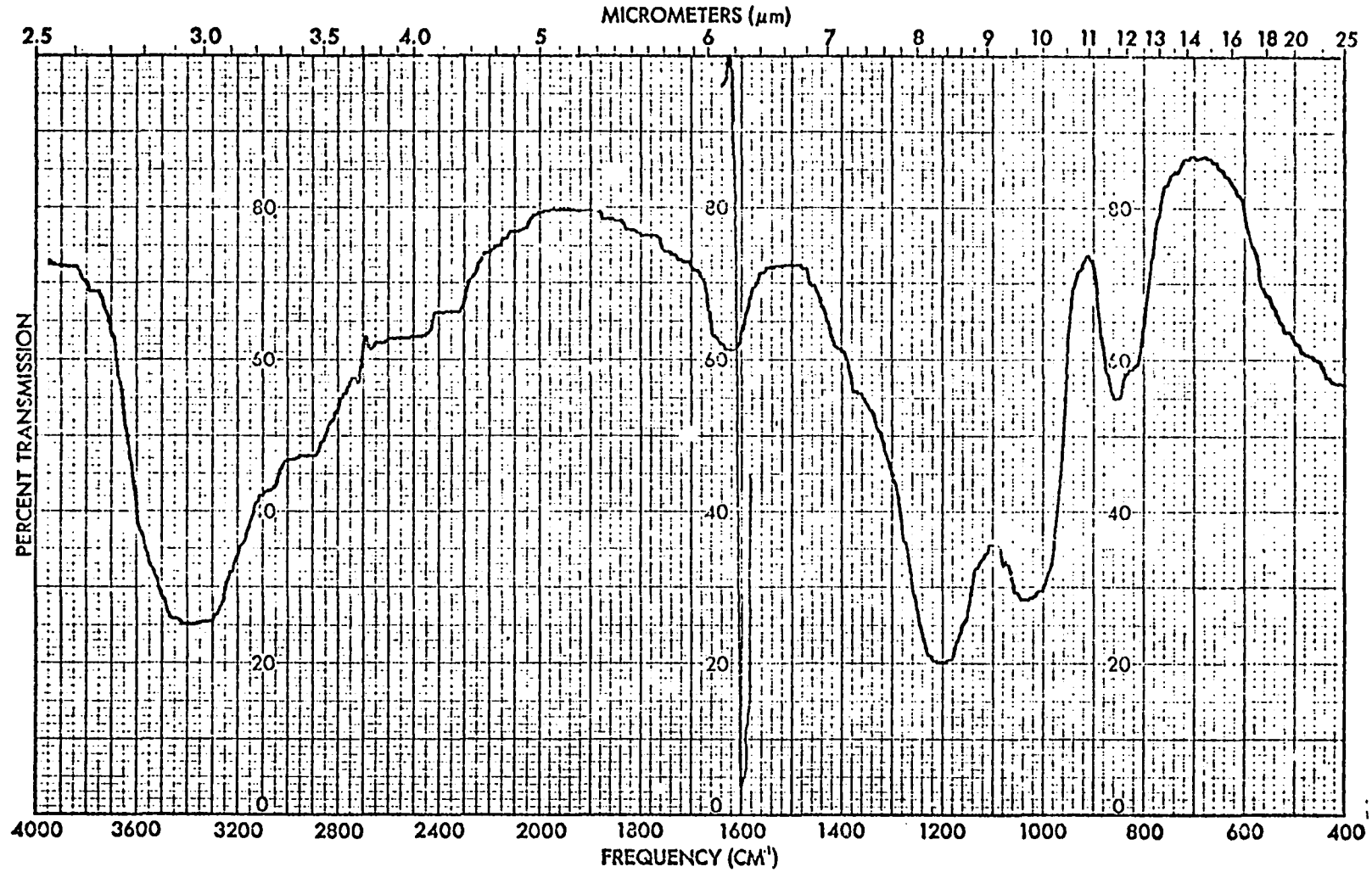

Figure A3. Infrared Spectra of Sulfated Kraft Paper (Acid Form).

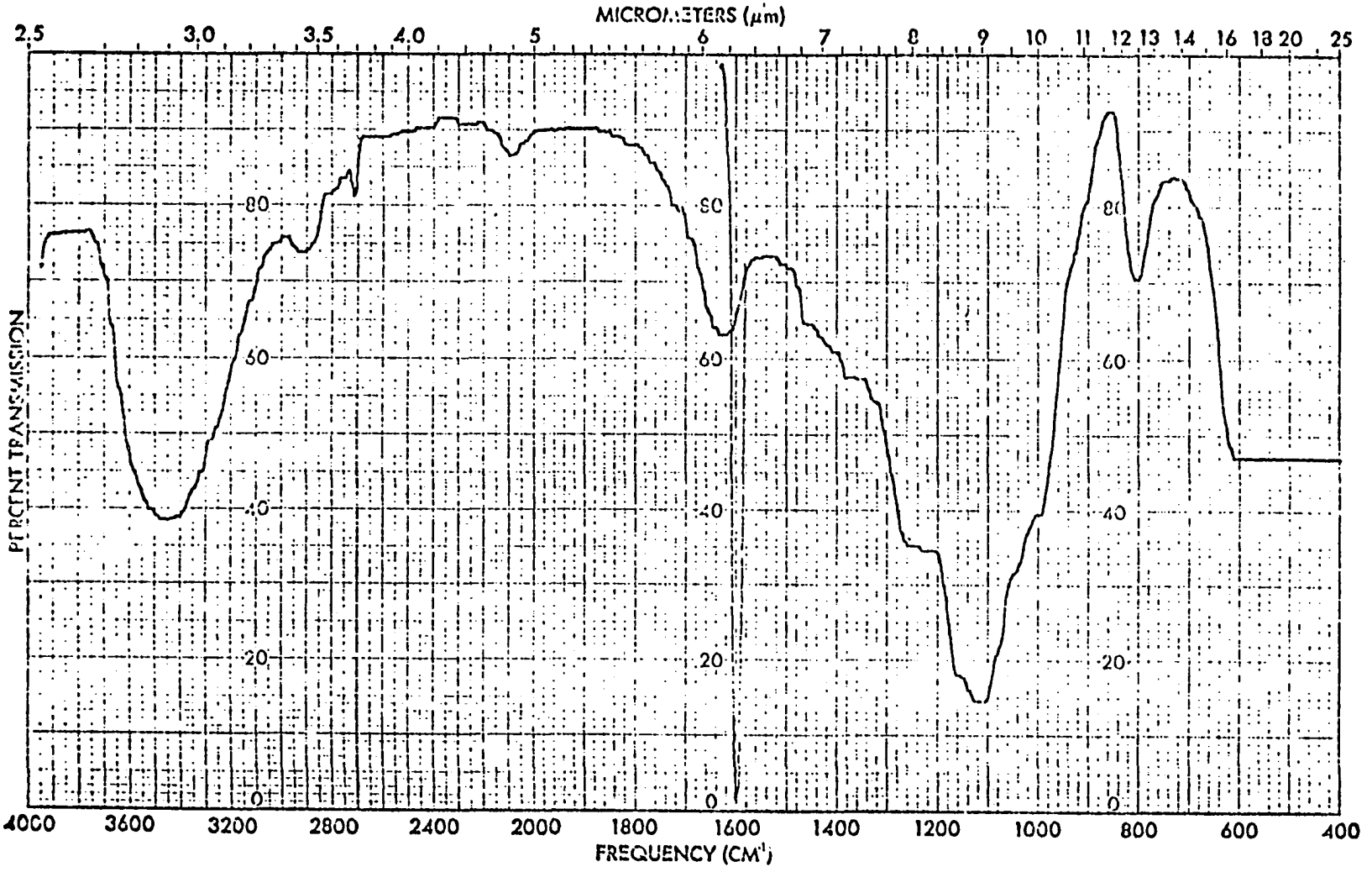

Figure A4. Infrared Spectra of Sulfated Newsprint Paper(Acid Form).