Korean J. Chem. Eng., **16**(5), 576-580 (1999)

Effect of Chemical Modification of Carboxyl Groups in Apple Residues on Metal Ion Binding

Sung Ho Lee†, Jong Sik Shon, Hongsuk Chung, Moo-Yeal Lee and Ji-Won Yang*

Korea Atomic Energy Research Institute, P.O. 105, Yusong, Taejon 305-600, Korea
*Bioprocess Engineering Research Center, Department of Chemical Engineering, Korea Advanced Institute of Science and Technology, Taejon 305-701, Korea
(*Received 17 April 1999 • accepted 5 July 1999*)

Abstract–AR (apple residue) was used as an alternative and cheap material for binding metal ions due to the presence of carboxyl and phenolic functional groups. The binding capacity of copper, lead, and cadmium by AR was pH dependent. Carboxyl groups of AR were esterified by acidic methanol to determine the contribution of carboxyl groups to metal ions binding. The extent of esterification was determined by analyzing the amount of methanol released in the sample hydrolysates by gas chromatography. The effect of esterification on binding metal ions was investigated in batch experiments by unmodified and modified AR. All esterified AR showed significant decreases in binding copper, lead and cadmium. The loss in the capacity of metal ion binding was proportional to the extent of esterification. The capacity of metal binding decreased with increase in the concentration of methanol in the respective hydrolysates or the modified AR. The data indicate that carboxyl groups on AR play an important role in the metal ion binding.

Key words : Apple Residue, Metal Binding, Chemical Modification, Carboxyl Groups, Heavy Metals

INTRODUCTION

The removal of toxic trace metals from aqueous solution is of great importance environmentally and industrially. A number of researches were performed for the extraction of toxic heavy metals from aquous solution [Tsezos and Volesky, 1981; Tsezos, 1983; Lujan et al., 1994; Reed, 1992; Gardea-Torresdey et al., 1996]. Recently, recovery and recycling of organic residues (agricultural, urban, fish industry, etc.) has become one of the main fields of investigation in developed countries due to their large waste volume [Randall and Hantala, 1975; Scott, 1992]. In the present study, apple residue (AR) from apple-juice process was used as an alternative and cheap polymeric material with suitable properties for removing metal ions due to the presence of acidic groups (carboxyl and phenolic group). To determine the binding mechanism of metal ions to AR surfaces, it is necessary to determine which chemical groups on AR are responsible for binding different metal ions. One approach to gaining this information is a chemical modification of the metal binding functional groups on the AR surface.

Gardea-Torresdey and coworker [Gardea-Torresdey et al., 1990] esterified the carboxyl groups on five different cells of algae with acidic methanol and investigated the effect of esterification of carboxyl groups for metal ions binding. They demonstrated that carboxyl groups on cell walls of algae are responsible for copper ion binding, but are not responsible in gold binding. Beverridge and Murray [1980] chemically modified amine and carboxyl groups on the cell wall of *Bacillus subtilis* to neutralize their electrochemical charge for determination of their contribution to the metal uptake process. Kuyucak and Volesky [1989] found that alginates of cell wall (containing carboxyl groups) of the seaweed *Ascophyllum nodosum* played an important role in cobalt ion binding.

In this study, the methanol esterification of carboxyl groups of AR was experimentally studied. An adaptation of the procedure used by Wilcox [Wilcox, 1972] was utilized to modify carboxyl groups with acidic methanol. The basic chemical reaction is shown below:

$$AR{-}C(O)O + CH_3OH{-}(HCl) \rightarrow AR{-}C(O)OCH_3 + H_2O$$

The extent of carboxyl group modification was monitored by subsequent analysis of methanol released after base hydrolysis of the esterified carboxyl groups. In addition, the effects of solution pH and esterification on metal ion binding to AR were investigated. The binding abilities of the modified AR for copper, lead and cadmium were determined and compared to that of unmodified AR.

MATERIALS AND METHODS

AR, which was obtained from an apple-juice processing factory, consists of processed skins, seeds and stems, and contains as much as 12% of the wet weight of original fruit. AR is mainly composed of cellulose (30%) and lignin (19%), both with a capacity for binding metal cations due to their carboxyl and phenolic groups. AR was dried overnight at 60 °C in a convection oven, ground by a ball mill, and sieved into different fractions. In order to eliminate soluble components such as tannins, resins, reducing sugars and colouring agents, the residues were successively washed with 0.5 N HCl and then with distilled deionized water until a constant pH was achieved. FTIR analy-

†To whom correspondence should be addressed.
E-mail : shlee6@nanum.kaeri.re.kr

sis was performed to identify functional groups of AR by FTIR spectrometer (Perkin-Elmer, Model 1725X).

Modification of carboxyl groups of AR with acidic methanol was carried out according to the procedure of Wilcox [1972]. Nine grams of AR (sieved to particle size of approximately 300 μm diameter) was suspended with continuous agitation in 633 ml of 99.9% methanol containing 5.4 ml of concentrated hydrochloric acid. Aliquots were removed from the suspension after 6, 24 and 48 hour at room temperature. The reactions were quenched by addition of a large volume of distilled deionized water. The samples were then dialyzed exhaustively against 0.001 M HCl in Spectra/Por molecular porous dialysis membrane at room temperature to eliminate unreacted methanol, and dialyzed against distilled deionized water to make samples at neutral pH and then were lyophilized to reduce volume. Modification of carboxyl groups of AR with methanol or hydrochloric acid was also carried out according to the procedure described previously.

The amount of modified carboxyl groups was determined according to the method of Torresdey et al. [Torresdey et al., 1990]. The extent of esterification of AR was examined by base hydrolysis of the modified AR and subsequent analysis of the released methanol by gas chromatography (GC). Base hydrolysis was performed by suspending 50 mg of AR (modified, unmodified and modified with only methanol or HCl) with 1.25 ml of 0.005 M sodium citrate in 0.1 M NaCl at pH 5.0. Subsequently, 0.1 ml of 1.0 M NaOH was added to the suspension. After being agitated for 5 min, samples were sealed with parafilm and were incubated overnight at 4 °C. Then, samples were handmixed until an even suspension was obtained and were centrifuged at 5,000 rpm for 15 min at 4 °C. The supernatant fraction was transferred into an eppendorf tube and mixed with 0.082 M sodium citrate at pH 3.0 to produce a final sodium citrate concentration of 0.0123 M. Samples were then placed in 5 ml Wheaton GC vials with teflon caps. One microliter of the resulting solution was then injected into a gas chromatograph (Shimadzu GC-14) equipped with an automated splitless injector, a flame ionization detector, and a 25 m, 0.25 mm i.d. phenylmethyl silicon fused capillary column (CBP5). The conditions for GC analysis were as follows: injector temperature, 250 °C; detector temperature, 275 °C; carrier gas N_2; carrier gas pressure, 1 kg/cm^2; carrier gas velocity, 32 ml/min. All analyses were performed in split mode. Samples were run isothermally at 40 °C for 5 min. Between analyses, the temperature of the column was increased to 220 °C at the rate of 10 °C/min and then returned to the original temperature, in order to evaporate H_2O in column. To confirm the methanol peak, 2-propanol was used as the chromatographic internal standard. The methanol concentration was determined by integrating the peak area using an integrator (a Shimadzu C-R6A chromaropac) and was compared with methanol standards.

Batch experiments were performed at room temperature using AR and modified AR to investigate the contribution of carboxyl group on metal ions binding. Samples were prepared in duplicate. All the glassware and polyethylene tubes were acid-cleaned and rinsed thoroughly before use with distilled deionized water. An initial metal concentration of 10 ppm was prepared by diluting 1,000 ppm of standard solution used for atomic absorption spectrophotometry (AAS). The 10 ml solution thus prepared was added to each 15 ml test tube containing pre-weighted unmodified and modified AR. pH of solution in test tubes was adjusted with 0.1 N/1 N NaOH and 0.1 N/1 N HCl, and then each test tube was sealed with a cap and placed on a rotary shaker (Roto-Torque model, model 7637). Test tubes were removed after shaking for 24 hours and centrifuged for 5 min at 3,000 rpm. The supernatant was analyzed by flame AAS (Perkin-Elmer, Model 3100) to determine residual metal content. Blank tests were also performed without AR to investigate the removal which might occur via metal precipitation and adsorption on the glass wall.

RESULTS AND DISCUSSION

1. FTIR Analysis Results on the Surface of AR

FTIR analysis was performed to identify functional groups of AR by FTIR spectrometer (Perkin-Elmer, Model 1725X). Bas-

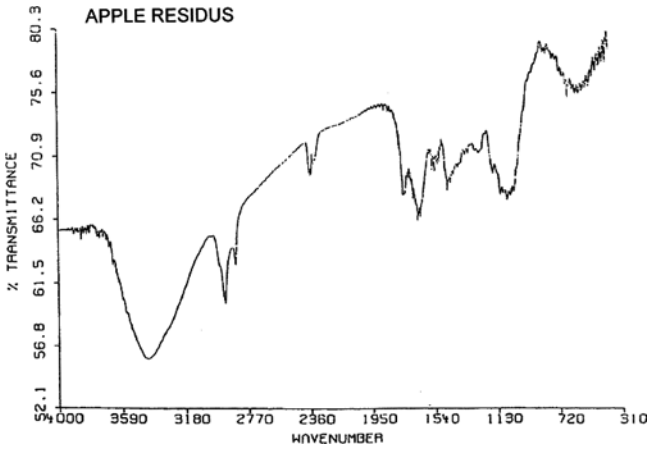

Fig. 1. FTIR analysis results on the surface of AR.

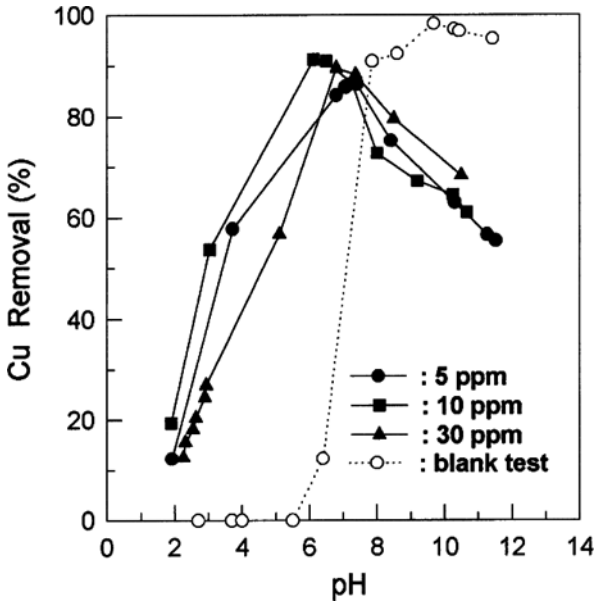

Fig. 2. Effect of pH on the removal of copper by AR.
[AR: 0.01 g/10 ml, I: 0.01 M NaCl]

al groups on AR were observed such as : (1) the peak at 3,400 cm^{-1}, probably OH^- band of phenolic functional groups, (2) the peak at 1,700 cm^{-1}, possibly C-O or C=O band of carboxylic groups, (3) the peak at 1,440 cm^{-1}, perhaps OH^- bond of carboxylic groups. Based on these analysis results, functional groups of AR were identified as carboxylic and phenolic groups. Similar analysis results of functional groups of AR have been reported by Maranon and Sastre [1991].

2. Effect of pH for Metal Binding

pH of solution has been identified as the most important variable which governs metal adsorption on hydrous solids. This is partly due to the fact that hydrogen ions themselves are strongly competing adsorbates, and the solution pH influences the speciation of metal ions and the ionization of surface functional groups [Snoeyink and Jenkin, 1980].

In Fig. 2, Fig. 3, and Fig. 4, the effect of pH for copper, lead and cadmium removal in ligand free system is illustrated. The optimal pH ranges for copper, lead and cadmium were from pH 5.5 to 7.0, pH 6.5 to 8.0, and pH 8.0 to 9.5, respectively. The maximum removal was 91.2% for copper, 95.3% for lead, and 91% for cadmium, respectively. Blank tests are also shown to verify that the removal mechanism is purely biosorption. As indicated in Fig. 2, precipitation of copper occurs at pH greater than 5.5. But, if precipitation does contribute to the removal mechanism, the removal capacity should not have decreased at pH greater than 7.5. The decrease in copper removal capacity at pH>7.5 may have been caused by the complexation of copper with hydroxide. As can also be seen in the results of blank tests of Fig. 2, precipitation does not contribute to the removal mechanism of lead and cadmium.

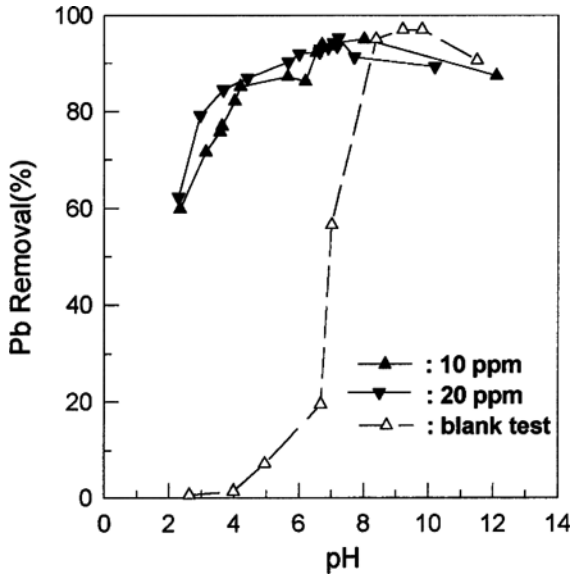

Fig. 3. Effect of pH on the removal of lead by AR.
[AR: 0.01 g/10 ml, I: 0.01 M NaCl]

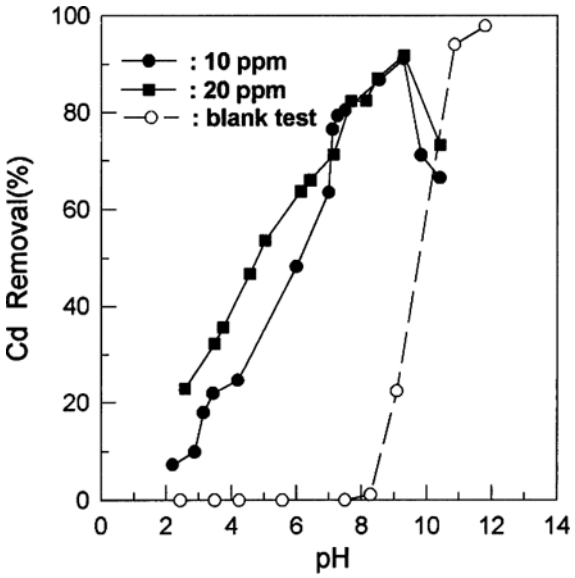

Fig. 4. Effect of pH on the removal of cadmium by AR.
[AR: 0.01 g/10 ml, I: 0.01 M NaCl]

3. Chemical Modification and Extent of Esterification

Table 1 displays the concentrations of methanol determined in hydrolysates of modified AR. The concentration of methanol detected in the respective hydrolysates increases rapidly

Table 1. Gas chromatographic analysis of methanol released from esterified AR

Reaction type	Hours modified	Methanol release (mmol/g AR)
Reaction with MeOH-HCl	0 hr	0.000±0.000
	6 hr	0.304±0.003
	24 hr	0.317±0.006
	48 hr	0.489±0.003
Reaction with MeOH	48 hr	0.000±0.000
Reaction with HCl	48 hr	0.000±0.000

*Each value represents mean ± standard deviation of four replicated analyses

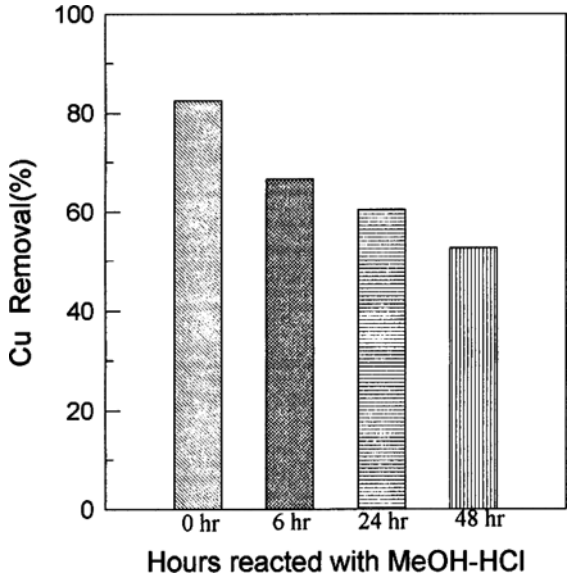

Fig. 5. Effect of acidic methanol modification on copper binding.
[I: 0.1 N NaCl, pH: 5.7]

during the first 6 hr of esterification and then levels off so that only approximately 2.7% additional carboxyls were esterified between 6 hr and 24 hr of reaction time, and additional (about 35.2%) carboxyls were esterified between 24 hr and 48 hr of reaction time. No methanol is detected in the hydrolysate of the unesterified control. In addition, AR equlibrated with methanol alone and/or only hydrochloric acid for 48 hr showed no methanol present in sample hydrolysates.

4. Effect of Esterification on Metal Binding

Fig. 5 shows the effect of esterification on copper binding at pH 5.7. The capacity of copper binding by unmodified AR was 82.5%. However, it decreases significantly by esterification of carboxyl groups of AR, and the extent of loss of copper binding capacity was proportional to the extent of esterification. As the concentration of methanol found in the respective hydrosates for most modified AR increases, copper removal capacity decreases. AR esterified for 6 hr, 24 hr, and 48 hr exhibit about 15.9%, 20.1%, and 30% decreases in copper binding, respectively. These experimental results show a similar trend to the results of Gardea-Torresdey and coworkers [Gardea-Torresdey et al., 1990]. They investigated the effect of esterification of carboxyl groups of five different algal biomass for metal ions binding, and reported that uptake of copper by algal biomass decreased dramatically by esterification of carboxyl groups, and the biomasses of *Chlorella*, *Spirulina*, *Eisenia*, *Laminaria*, and *Cyanidium* esterified for 48 hr exhibit 66%, 57%, 27%, 40% and 46% decreases in copper binding, respectively.

The effect of esterification on lead binding at pH 5.5 is shown in Fig. 6. Similar to the experimental result for copper binding, the degree of loss of lead binding capacity was proportional to the extent of esterification of AR. The capacity of lead binding by unmodified AR was 92.6%. However, the loss of lead binding capacity of 6 hr, 24 hr, and 48 hr esterified AR was 12.4%, 16.6%, and 38.8%, respectively.

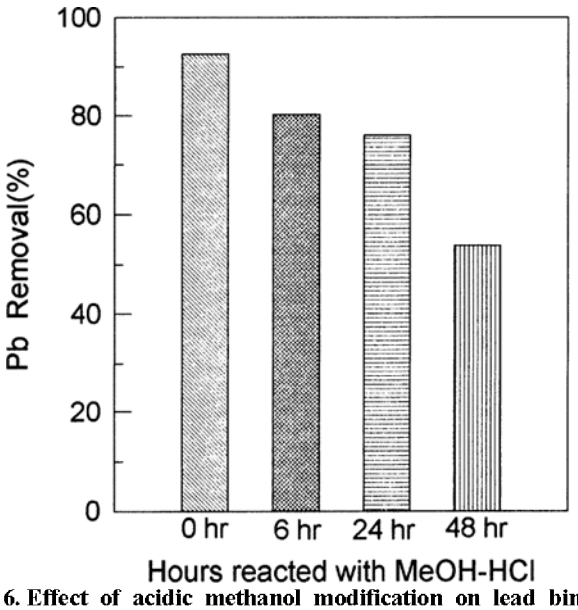

Fig. 6. Effect of acidic methanol modification on lead binding.
[I: 0.1 N $NaNO_3$, pH: 5.5]

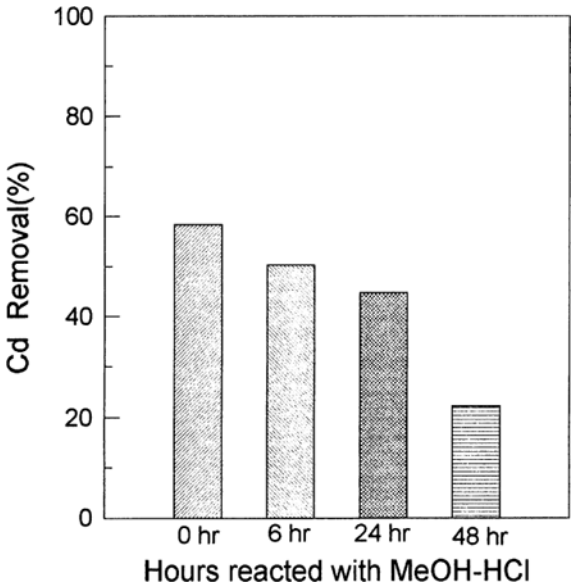

Fig. 7. Effect of acidic methanol modification on cadmium binding.
[I: 0.1 N NaCl, pH: 7.7]

Fig. 7 shows the effect of esterification on cadmium binding at pH 7.7. It also shows similar experimental results as copper and lead binding. The capacity of cadmium binding by unmodified AR was 58.4%. But, AR esterified for 6 hr, 24 hr, and 48 hr exhibit 8.1%, 13.7%, and 36.1% decreases in cadmium binding, respectively. These experimental results indicate a good correlation between the loss of metal binding capacity and the amount of methanol released by hydrolysis (Table 1). As the concentration of methanol found in the respective hydrosates for most modified AR increases, metal binding capacity decreases. From these experimental results, carboxyl groups on AR may provide major sites for metal ion binding.

CONCLUSIONS

AR was used as an alternative and cheap polymeric material for removing metal cations due to the presence of carboxyl and phenolic functional groups. The removal capacity of copper, lead, and cadmium by AR was pH dependent. The optimal pH ranges for copper, lead and cadmium were from pH 5.5 to 7, pH 6.5 to 8.0, and pH 8.0 to 9.5, respectively. The maximum removal was 91.2% for copper, 95.3% for lead, and 91% for cadmium. However, the chemical modification of carboxyl group of AR resulted in a decrease in binding of copper, lead and cadmium. The degree of loss of metal binding capacity is proportional to the extent of esterification of AR. The 48 hr esterified AR exhibit 30%, 38.8% and 36.1% decreases in copper, lead and cadmium binding, respectively. These experimental results indicate a good correlation between the loss of metal binding capacity and the amount of methanol released by hydrolysis. As the concentration of methanol found in the respective hydrolysates for most modified AR, metal binding capacity decreases. From these experimental results, it was found that carboxyl groups on AR play an important role in metal

ions binding.

ACKNOWLEDGEMENT

This project has been carried out under the Nuclear R & D Program by MOST.

REFERENCES

Beveridge, T. J. and Murray, R. G. E., "Sites of Metal Deposition in the Cell Wall of *Bacillus subtilis*," *J. Bacteriol.*, **141**, 876 (1980).

Gardea-Torresdey, J. L., Becker-Hapak, M. K. and Darnall, D. W., "Effect of Chemical Modification of Algal Carboxyl Groups on Metal Ion Binding," *Environ. Sci. Technol.*, **24**, 9 (1990).

Gardea-Torresdey, J. L., Tiemann, K. J. and Gonzalez, J. H., "Uptake of Copper Ions from Solution by Different Populations of Mediccago Sativa," *Sep. Sci. Technol.*, **14**(1), 119 (1996).

Kuyucak, N. and Volesky, B., "Accumulation of Cobalt by Marine Algae," *Biotechnol. Bioeng.*, **33**, 809 (1989).

Lujan, J. R., Darnall, D. W., Stark, P. C., Rayson, G. D. and Gardea-Torresdey, J. L., "Metal Ion Binding by Algae and Higher Plant Tissues," *Solvent Extr. Ion Exch.*, **12**(4), 803 (1994).

Maranon, E. and Sastre, H., "Heavy Metal Removal in Packed Beds using Apple Wastes," *Biores. Technol.*, **38**, 39 (1991).

Reed, B. E., "Metal Adsorption by Activated Carbon," *Sep. Sci. and Tech.*, **27**(14), 1985 (1992).

Randall, J. M. and Hantala, E., "Removing Heavy Metal Ions from Waste," U. S. Patent 3,925,192 (1975).

Scott, C. D., "Removal of Dissolved Metals by Plant Tissue," *Biotechnol. Bioeng.*, **39**, 164 (1992).

Snoeyink, V. L. and Jenkin, D., "Water Chemistry," John Wiley & Sons, New York (1980).

Tsezos, M., "The Role of Chitin Uranium Adsorption by R. arrhizus," *Biotechnol. Bioeng.*, **25**, 2025 (1983).

Tsezos, M. and Volesky, B., "Biosorption of Uranium and Torium," *Biotechnol. Bioeng.*, **23**, 583 (1981).

Wilcox, P., "The Esterification of Carboxyl Groups by Acidic Methanol," *Methods enzymology*, **25**, 596 (1972).