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Purification of Carbohydrate-Containing Substrates from Plant Biomass Hydrolysates

**YURI I. KHOLKIN,[1] VLADIMIR V. VIGLAZOV,[1]
VLADIMIR B. KIND,[1] AND HOWARD D. METTEE*,[2]**

*[1]St. Petersburg Forest Technical Academy, Institutsky Per., 5, 194021,
St. Petersburg, Russia; and [2]Youngstown State University,
One University Plaza, Youngstown, OH 44555,
E-mail: hdmettee@cc.ysu.edu*

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Abstract

Carbohydrate-containing substrates are produced in Russia by means of dilute acid hydrolysis of wood and vegetable residues. Hydrolysates contain 3 to 4% monosaccharides and nearly 1% impurities. The common schemes used to prepare the hydrolysate for fermentation include cooling by self-evaporation; inversion of oligosaccharides; neutralization of sulfuric (and organic) acids; addition of mineral food substances containing nutrient N, P, and K salts; aeration to coagulate colloidal substances; and sedimentation of residues. Our work led to the suggestion that a flocculation step using cationic polyelectrolytes be added to purify more completely the substrates from lignin-huminic substances (LHS). This method permits the transfer of a part of the dissolved and colloidal LHS into a suspended state and the removal of more than 90% of the suspended materials, thereby increasing the yield and quality of ethanol and fodder yeast. Three industrial plants have reported successful results using this method in the past few years. Flocculation has also proven itself capable of clearing waste culture liquids of their dispersed phases by extents of 30–60%, as measured by chemical oxygen demand, color depth, or lignin content parameters, prior to consideration of such wastewater for recycling.

Index Entries: Flocculation; vegetable hydrolysates; cationic polymers; purification.

Introduction

Previously we studied the electrosurface properties of lignin-huminic substances (LHS) *(1)*. Electrophoresis fractionation of LHS was made by

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*Author to whom all correspondence and reprint requests should be addressed.

preparative isoelectric focusing. It produced more than 10 colored fractions of LHS and their measured isoelectric points (p*I*s). The majority of these fractions had p*I*s in the range of 3.5–6.5. Therefore, the main part of LHS dissociates as macroanions and forms negatively charged colloidal substances. On the other hand, the cells of asporogenous yeast grown in a pure cultural liquid have a positive charge; that is, ζ-potential is nearly 40 mV for *Candida scottii*, a commonly used industrial strain. Owing to electrostatic forces, yeast cell surfaces adsorb LHS, which causes difficulties in mass transfer through cell membrane. We proposed that cationic polyelectrolytes would be the most efficient flocculant for LHS, owing to electrostatic interaction between oppositely charged polymolecules. To demonstrate the efficiency of this method, an experimental study, tested under industrial conditions, was conducted.

Materials and Methods

Raw Material

Industrial acid hydrolysates of softwood and vegetable wastes (mixtures of corncob, rice husk, and cotton hulls) were obtained under common industrial conditions by the percolation method (180–190°C, 0.5–1% H_2SO_4, acid solution/softwood ratio 16). Hydrolysates of softwood were neutralized by water solutions of ammonia up to pH 4.1, and the hydrolysates of vegetable wastes were neutralized by milk of lime up to pH 3.0, and then to pH 4.1 by ammonia.

Polyelectrolytes

Poly(diallyl-dimethyl ammonium chloride) (PDADMAC), an industrial-grade sample, was obtained from Kaustik, Sterlitamak, Russia, and poly(4-vinyl-benzyl-trimethyl ammonium chloride) (PVBTMAC), an industrial-grade sample, was obtained from Karbolit, Kemerovo, Russia. Table 1 gives their characteristics.

For addition to hydrolysates, the polyelectrolytes were diluted by distilled water to 0.1%.

Flocculation

For flocculation experiments, we used a simple apparatus. Different volumes of polyelectrolyte solutions were added to the neutralized hydrolysates. After magnetic stirring for 30 s at controlled temperature, the hydrolysate stood for 20 min. Then it was filtered through a paper filter, and the optical density (OD) of filtrate, mass of the cake, and filtration velocity were measured.

For measuring the settling speed, this same process was carried out in the cylinder under controlled temperature. When stirring stopped, samples were taken from the top part of the cylinder at the same level, at definite times during 1 h. The mass of dispersed substance was determined in samples after filtering.

Table 1
Characteristics of Cationic Polyelectrolytes[a]

Polymer	[η][b] (dL/g)	M_W (10^3)	ζ (mV)	EC (mg-eqv/g)
PDADMAC	1.80	300	41	4.0
PVBTMAC	0.13	100	37	3.5

[a] [η], intrinsic viscosity; M_W, weight average of molar mass of the polymers; ζ, ζ-potential; EC, exchange capacity.

[b] Determined at 20°C in 0.1 *M* NaCl.

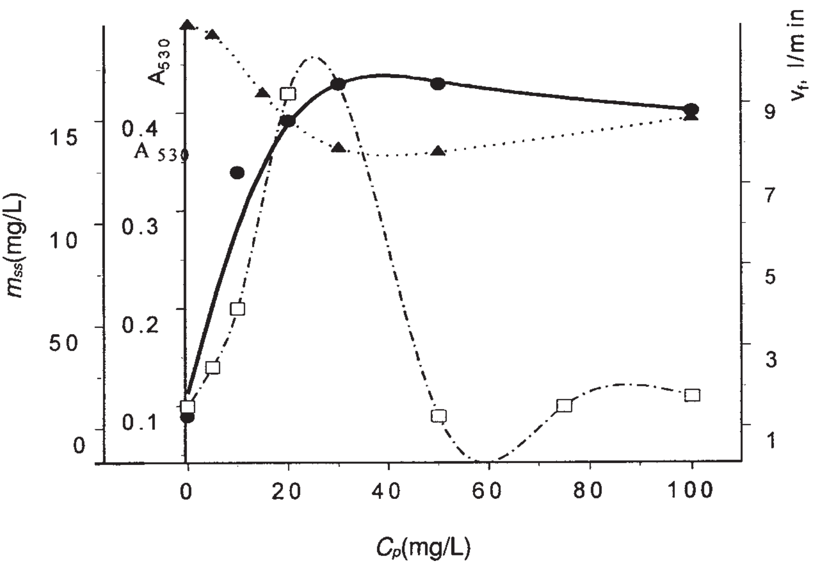

Fig. 1. Flocculation of vegetable wastes hydrolysate by PDADMAC at 80°C and pH 4.2. (—●—), m_{ss} (mg/L); (· · ▲ · ·), A_{530}; (· – □ – ·), v_f (L/min).

Results and Discussion

To establish the effectiveness of flocculation for the purifying of hydrolysates, The mass of dissolved and colloidal LHS transferred into solid phase (m_{ss}) was determined during the flocculation process at different levels of polyelectrolytes (C_p). In these experiments, changes of OD (A_{530}) and filtering velocity (v_f) were measured (Fig. 1). These data show that the region of optimal concentrations (rates) of flocculant exists where the maximum destabilization of the disperse system of the hydrolysate takes place. At concentrations of PDADMAC of 20–30 mg/L, extrema of the determined characteristics were observed.

Comparison of the mass of dissolved and colloidal LHS transferred into the solid phase (m_{ss}), and the changes of OD (A_{530}) during the flocculation process at different levels of cationic polyelectrolytes and nonionic water-soluble polymer poly(acrylamide) (PAA), shows that cationic polyelectrolytes with a high density of positive charge in the polymer

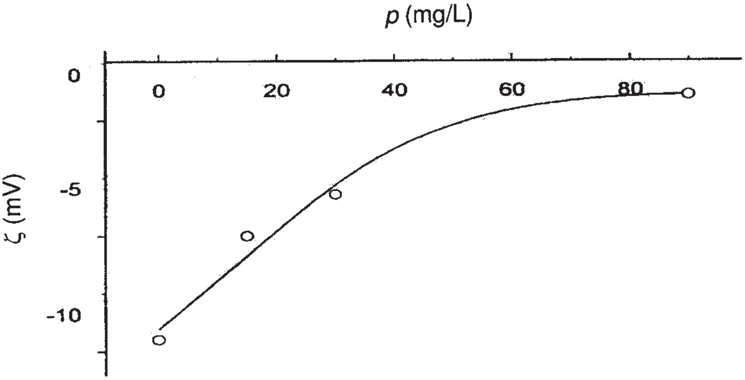

Fig. 2. Dependence of ζ-potential of LHS from concentration of PDADMAC.

Table 2
Efficiency of Purification of Hydrolysate
by Cationic Polyelectrolytes and PAA (pH 4.2, 80°C)

PDADMAC			PVBTMAC			PAA[a]		
C_p (mg/L)	m_{ss} (mg/L)	A_{530}	C_p (mg/L)	m_{ss} (mg/L)	A_{530}	C_p (mg/L)	m_{ss} (mg/L)	A_{530}
0.0	40	0.21	0.0	40	0.21	0.0	40	0.21
5.0	64	0.20	5.0	71	0.22	0.7	45	0.22
10.0	56	0.18	10.0	82	0.20	2.0	20	0.22
30.0	125	0.17	30.0	115	0.16	10.0	25	0.24
50.0	126	0.16	50.0	135	0.13	30.0	96	0.27

[a] PAA at concentrations more than 30 mg/L stabilizes disperse system of hydrolysate and no flocculation is observed.

chain are significantly more efficient for purification than nonionic polymers with large molecular masses (about 1,000,000). This strongly suggests that the flocculation of hydrolysates of plant raw materials has mainly a charge neutralization mechanism, and in a lesser degree a bridging mechanism *(2,3)*.

Measurement of the ζ-potential of LHS shows that the surface charge depends on the concentration of PDADMAC (Fig. 2). It decreases from –11.4 to –5.2 mV after addition of the first 30 mg/L of polymer. Then it changes more slowly—from 5.2 to 0.7 mV after the addition of the next portions of PDADMAC from 30 to 90 mg/L. These data show that the maximum efficiency of flocculation occurs at 15–30 mg/L (Fig. 1), which also corresponds to reducing the surface charge of LHS approximately two times. This indicates that flocculation has taken place not just owing to simple charge neutralization, as in coagulation by low-molecular electrolytes. At the same time, the data in Table 2 indicate that nonionic high-molecular PAA also causes some flocculation. Evidently the adsorption of long-chain polymers on several particles of LHS ("bridge" mechanism of

Table 3
Industrial Results

Month	Average content of protein in yeast (%)		Increase in protein content (%)	Average content of ash elements in yeast (%)		Decrease in ash in yeast (%)
	Basic year	Year of experiment		Basic year	Year of experiment	
June	41.34	43.60	2.26	8.8	8.0	0.8
July	40.26	43.40	3.14	8.5	8.0	0.5
August	41.47	42.50	1.03	8.7	8.2	0.5
September	40.60	42.86	2.26	8.5	7.9	0.6
October	40.41	43.67	3.26	8.2	7.7	0.5
November	39.24	44.02	4.78	8.6	6.9	1.7
December	41.75	43.70	1.95	8.2	6.7	1.5
January	41.20	43.14	1.94	8.1	7.5	0.6
February	40.48	43.97	3.49	8.6	7.6	1.0
March	40.61	44.38	3.77	8.1	7.8	0.3
April	41.30	44.35	3.05	8.3	7.9	0.4
May	41.60	44.09	2.49	8.0	7.8	0.2
Average	40.86	43.64	2.78	8.4	7.7	0.7

flocculation) is taking place. Therefore, at a decrease in ζ-potential of LHS of approximately two times, a strong flocculation (e.g., large flocks, high velocity of sedimentation, and good clarification) is obtained *(4)*.

Industrial Experiment

The results of the laboratory experiments were confirmed in an industrial setting over a long-time, full-scale experiment. It was carried out in a plant producing 20,000 metric tons of fodder yeast from hydrolysate vegetable wastes. No other changes in technology were made during the reviewed period. PDADMAC was used as flocculant. A water solution of polymer diluted to 1% was added to neutralize the hydrolysate at optimal values of temperature and pH, and the level of PDADMAC was estimated at laboratory conditions. No other changes—raw material content, hydrolysis, or microbiological technology—were made during the experimental period (1 yr), which was compared to a base period (1 yr before the experiment).

PDADMAC conglomerates colloidal, soluble LHS and dispersed gypsum, created during neutralization of H_2SO_4 by lime, and removes these contaminations as large-sized flocs, which easily sediment in settling tanks. The settling speed of the disperse phase increased 6–24 times. The concentration of dispersed substances in cultural liquid decreased from 0.5 to 0.04–0.05 g/L. The general results of the industrial experiment show that flocculation caused an increase in the protein concentration in fodder yeast (Table 3). This is very important because protein is a target substance in

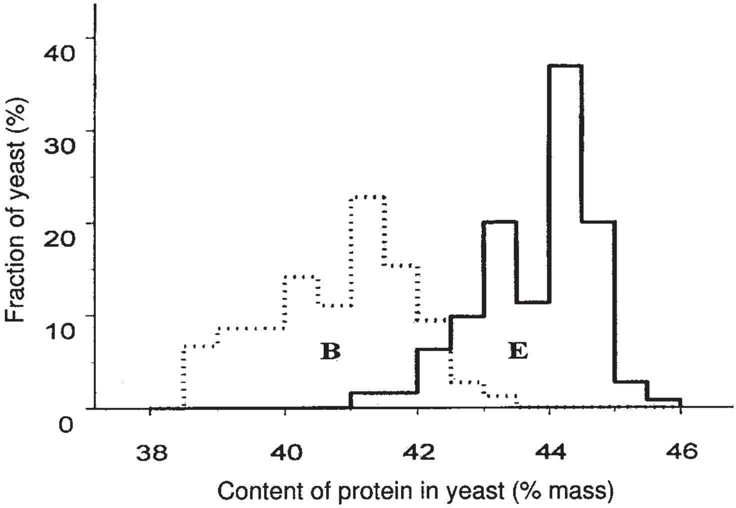

Fig. 3. Distribution of food yeast according to their protein content. B, Base year; E, year of experiment.

food yeast *(5)*. The average increase in protein concentration was 2.8%. The rate of yeast action on the reducing sugars of the hydrolysate increased from 42.7% (year before the experiment) to 43.5% (year of the experiment). These differences on the industrial scale were reflected by the 3.6% increase in protein content on the benchscale, whereas the rate of yeast action on the reducing sugars had increased more dramatically from 45.8 to 52.0%. Figure 3 shows the distribution of food yeasts according their protein content.

At the optimal level of polymer content, no residual unbound PDAMAC was determined in the purified substrate *(6)*. The flocculation method provided significant technical and economic benefits on the industrial scale.

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