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# Affinity Foam Fractionation of *Trichoderma* Cellulase

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## Abstract

Cellulase could not be selectively collected from fermentation broth by simple foam fractionation, because of the presence of other more surface-active compounds. A new approach of affinity foam fractionation was investigated for improvement. A hardwood hydrolysate (containing cellulose oligomers, substrates to cellulase) and two substrate analogs, i.e., carboxymethyl cellulose (CMC) and xylan hydrolysate, were added before the foaming process. The substrates and substrate analogs were indeed found to bind the cellulase selectively and form more hydrophobic complexes that partition more readily onto bubble surfaces. In this study, the effects of the type and concentration of substrate/analog as well as the presence of cells at different growth stages were examined. The foam fractionation properties evaluated included foaming speed, foam stability, foamate volume, and enrichment of filter paper unit (FPU) and individual cellulase components (i.e., endoglucanases, exoglucanases, and β-glucosidases). Depending on the broth and substrate/analog employed, the foamate FPU could be more than fourfold higher than the starting broth FPU. Addition of substrate/analog also deterred the enrichment of other extracellular proteins, resulting in the desired cellulase purification in the foamate. The value of E/P (enzyme activity-FPU/g/L of proteins) in the foamate reached as high as 18, from a lactose-based fermentation broth with original E/P of 5.6. Among cellulase components, exoglucanases were enriched the most and β-glucosidases the least. The study with CMC of different molecular weights (MW) and degrees of substitution (DS) indicated that the CMC with low DS and high MW performed better in cellulase foam fractionation.

**Index Entries:** Affinity foam fractionation; carboxymethyl cellulose; cellulose; cellulose hydrolysate; xylan hydrolysate.

## Introduction

Foam fractionation is a simple, inexpensive, environment-friendly process for protein concentration, and purification. Proteins, which have both hydrophobic and hydrophilic moieties, are surface-active and easily collectable from aqueous solutions by foam *(1,2)*. In addition, proteins of different surface hydrophobicity are expected to have different tendencies

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for adsorption on bubble surface, which provides the opportunity for simple protein separation or fractionation. The simple foam fractionation has a significant potential of reducing the cost of protein recovery in the pharmaceutical and food industries (3). Simple foam fractionation, however, cannot be applied to the products that do not have the highest efficiency in partitioning onto bubble/foam surfaces, among all of the materials present in the product-bearing broth. Cellulase separation from its fermentation broth belongs to this category.

Cellulase is a group of enzymes that hydrolyzes cellulose to glucose. Economic, effective cellulase production is critical to utilization of the abundant cellulosic materials as renewable feedstock. Cellulase is typically considered to contain three component groups (4). Endoglucanases attack insoluble cellulose in the middle of the chain and produce oligosaccharides with more than six glucose units. Exoglucanases nick the end of oligosaccharides to generate cellobiose (dimer). β-Glucosidases cleave cellobiose to two glucose molecules.

Foaming occurred in the cellulase fermentation, particularly during the stationary phase. Because the increase in foaming intensity appeared to parallel the profile of cellulase production, cellulase had been assumed to be the cause of foaming. More recent studies, however, provided evidence against the above assumption (5). In particular, when applying foam fractionation to separate cellulase from the fermentation broth, Zhang et al. (6) found that cellulase was surface-active but not the strongest in the broth, and, therefore, could not be selectively foamed out. None of the individual cellulase components, i.e., endoglucanases, exoglucanases, and β-glucosidases, showed higher activities in the collected foamate than in the original broth (6).

Enzymes have selective binding affinity to their substrates, substrate analogs, and compounds containing moieties of the substrates or analogs. The affinity binding of these agents with the active sites of enzymes also helps to protect the enzymes from being deactivated during the foaming process. Protein deactivation in foaming resulted mainly from surface denaturation, while other potential factors such as shear stress and oxidation did not contribute significantly (7,8). To enhance foam fractionation of cellulase from the fermentation broth, the effects of adding the substrate (hardwood hydrolysate) and different substrate analogs, including various types of carboxymethyl cellulose (CMC) and a xylan hydrolysate (XH), were examined in this study. Cellulase was reported to hydrolyze both CMC and xylan (9–14), indicating the existence of certain binding affinity of cellulase to these substrate analogs.

## Materials and Methods

### Fermentation

*Trichoderma reesei* Rut C-30 (NRRL 11460) was obtained from US Department of Agriculture (Agricultural Research Service Patent Culture

Collection, Peoria, Illinois). The culture was maintained at 4°C on slants of Potato Dextrose Agar (Sigma; 39 g/L, as recommended), with regular subculturing every 3–4 wk. The inoculum for fermentation, which provided the broth for foam fractionation study, was prepared by transferring three loops of cells from an agar slant to a 250-mL shake flask containing 50 mL of Potato Dextrose medium (Sigma, St. Louis, MO). After 2 d of cultivation at room temperature, the broth was added to a 2-L flask containing 500 mL of a defined medium modified from that used by Mandels and Weber *(15)*.

For cellulase production, the defined medium required not only C-substrates, as the source of material and energy for cell growth and maintenance, but also inducers, to activate the expression of all cellulase components. In this study, three medium systems were compared for their cellulase synthesis capacity and, more important to this study, for their foaming properties. The first medium included 5 g/L of glucose as C-substrate and 5 g/L of pure cellulose (Avicel, Sigma) as inducer and C-source (on hydrolysis by cellulase produced by cells). The second medium contained a hardwood hydrolysate, with 12–16 g/L of reducing sugars, preparation described elsewhere *(16)*, as both C-substrate and inducer. The above two media were used in batch fermentation. The broths used in the foaming study were generally harvested on the fifth day when the cellulase activity (assayed in FPU) reached the highest level. The third medium was lactose based, i.e., with lactose serving as both C-substrate and inducer. The fermentation with this medium was conducted in a batch-then-continuous mode. The original medium had 10 g/L of lactose; the feed for continuous culture had 20 g/L of lactose. The culture was grown to late exponential-growth phase and then converted to the continuous culture, with the feed rate computer controlled according to a pH-based algorithm, details described elsewhere *(17)*. The broth used in the foaming study was collected after about 1 wk into the continuous culture.

To avoid foaming, which would otherwise necessitate antifoam addition, surface aeration at the rate of 1 vvm was employed for oxygen supply into the broth that was magnetically stirred at the rate of 250 rpm. The fermentation was kept at room temperature (24 ± 1°C). Samples were taken daily. Cell-free media were used in most of the foaming experiments. In this case, the harvested fermentation broth was centrifuged at 8000 rpm (9300*g*) (Sorvall RC 5C Plus Superspeed Centrifuge, Sorvall, Newtown, CT) for 10 min to remove the biomass. The supernatant was collected for the subsequent affinity foaming study.

### *Affinity Foam Fractionation Study*

The experiments were conducted in 250-mL volumetric cylinders. The volume of each liquid sample used was 40 mL. An air diffuser, placed at the bottom of the cylinder, was used to generate fine bubbles for the foaming study. The total volume before foaming, including both the liquid sample and the air stone, was 50 mL. The air bubbling rate was kept at 1 vvm

(i.e., 40 mL/min) using a flow meter with a three-way valve. On bubbling, the foam rose and typically reached the maximal level within 5 min. The total foam volume was recorded. The bubbling was then stopped to allow the foam to collapse. The collapsing rate was also recorded as an indicator of the foam stability. The air bubbling was then resumed. While maintaining the foam at the highest level, the liquid broth remaining at the bottom (residue) was collected and its volume ($V_r$) measured. The foam in the foaming cylinder was next collapsed (if necessary, by blowing an air stream on the foam surface), and the cylinder wall and the air stone were rinsed with a known volume of deionized water ($V_w$). The diluted foamate was collected for analysis. The "actual" foamate volume ($V_f$) was obtained by subtracting $V_r$ from the initial sample volume (40 mL). The dilution factor, $(1 + V_w/V_f)$, was used to adjust all the analysis concentrations obtained with the diluted foamate.

## Analytical Methods

### *Reducing Sugar Concentration*

The reducing sugar concentration was measured by the nonspecific dinitrosalicylic acid (DNS) method, based on the color formation of DNS reagent when heated in the presence of reducing sugars *(18)*. The DNS reagent was prepared by dissolving 10 g of 3,5-dinitrosalicylic acid in 400 mL distilled water, adding 200 mL of 2 *M* NaOH, and then diluting the solution to a total volume of 1 L with distilled water.

### *Cell Concentration*

Because of the presence of cellulose in some systems, cell dry-weight concentration could not always be measured directly. Intracellular protein concentration was measured instead *(19)*, as described later. By centrifugation, the solids in broth samples were collected and washed twice with distilled water. The cells were then lysed in 3 mL of 0.2 *N* NaOH, at 100°C for 20 min. The protein concentration of the lysate was then measured by the standard Lowry method. The absorbance at 595 nm was measured with a UV/VIS spectrophotometer (Perkin-Elmer Lambda 3B). To establish the relationship between the intracellular protein concentration and cell dry-weight concentration, batch fermentation was made with glucose as the sole carbon source. Samples taken at different stages of the fermentation were analyzed for both cell dry-weight concentration and intracellular protein concentration. The relationship was established as:

$$\text{Cell dry-weight concentration (g/L)} = \text{intracellular protein concentration (g/L)} \times 8\ (\pm 0.5)$$

### *Cellulase Activity*

The total activity of cellulase was measured by the standard filter paper assay method *(15)*. Assays for the activity of individual enzyme

components, i.e., endoglucanases, exoglucanases, and β-glucosidases are briefly described here.

### Endoglucanases

A modified method of Berghem and Petterson *(20,21)* was used. A 1% CMC solution was prepared in 0.05 *M* sodium acetate buffer (pH 5.0). The CMC solution was incubated with 0.28 mL of the test enzyme solution at 50°C for 30 min. Almost 3 mL of 1% DNS reagent were added to terminate the reaction. The reducing sugar concentration produced from the enzymatic reaction was then measured and used to calculate the endoglucanase activity according to the following equation *(19,20)*.

$$\text{Endoglucanase activity (U/mL)} = \text{reducing sugars released (mg)} \times 0.66$$

### Exoglucanases

A modified method of Berghem and Petterson *(20,21)* was used. Almost 1 mL of the test enzyme solution was added to 1 mL of 2% Avicel suspension prepared in 0.05 *M* sodium acetate buffer (pH 5.0). After 30 min incubation at 40°C, 3 mL of 1% DNS reagent was added to end the reaction and the resultant reducing sugar concentration was measured. The exoglucanase activity was calculated according to the following equation *(22)*.

$$\text{Exoglucanase activity (U/mL)} = \text{reducing sugars released (mg)} \times 0.185$$

### β-Glucosidases

Three test tubes were used *(23)*. The test tube for cellobiose blank contained 1 mL each of 15 m*M* cellobiose solution, citrate buffer (pH 4.8), and water. A second test tube, for the sample blank, contained 1 mL sample and 2 mL water. The third tube, for the test sample, contained 1 mL each of the cellobiose solution, buffer, and the test sample. The test tubes were mixed, capped tightly, and incubated at 50°C for 30 min. Again, 3 mL of the DNS reagent were added and the resultant reducing sugar (glucose) concentration was measured by the DNS method. The absorbance of the sample, subtracted by those of the sample blank and the cellobiose blank, was used in determining the reducing sugar concentration. The β-glucosidase activity was determined according to the following equation *(22)*.

$$\text{β-Glucosidase activity (U/mL)} = \text{glucose released (mg)} \times 0.0926$$

## Results and Discussion

The presentation below is organized in three sections according to the different foaming agents added, i.e., cellulose (hardwood) hydrolysate (CH), CMC, and XH. The cross comparison between these foaming agents is included in the proper sections.

Table 1
Effects of Addition of Cellulose Hydrolysate and Cells of Different Culture Stages on Foaming Behaviors

| | | Foaming phase | | Holding phase | |
|---|---|---|---|---|---|
| Cells | Hydrolysate (%) | Volume (mL) | Time (s)[a] | Volume (mL) | Time (s)[b] |
| None | 0 | 250 | 153 | 250 | 393 |
| | 5 | 250 | 246 | 250 | 390 |
| Growth | 0 | 190 | 360 | 190 | 246 |
| phase | 5 | 250 | 311 | 250 | 310 |
| Stationary | 0 | 230 | 335 | 210 | 282 |
| phase | 5 | 180 | 351 | 160 | 351 |

[a]Time required in the foaming phase, to reach the maximum foam volume reported in the previous column.
[b]Time observed in the holding phase (with air bubbling being turned off), for the foam volume to change from the maximum volume to the volume reported in the previous column. In the first four systems, there were no foam volume changes observed during the time periods reported.

### *Affinity Foam Fractionation With Addition of Cellulose Hydrolysate*

Many foaming experiments were carried out. Only some of them are reported here to present the general, reproducible phenomena observed and the effects of important factors identified. The results from an experiment displaying the typical effects of three factors on the foaming behaviors are summarized in Tables 1 and 2. The factors were (1) the presence or absence of cells in the foaming broth; (2) the different growth stages of the cells present; and (3) the hydrolysate addition. The cells used in cell-containing systems were pre-grown in a glucose-based medium (with 10 g/L glucose). For studying the effects of different growth stages, the cells were harvested either at the late exponential growth phase (the third day of batch cultivation) or at the stationary phase (the fifth day). The broth supernatant used as the basal cellulase-bearing medium in all of the systems was prepared by a fermentation using the hydrolysate-based medium. The broth was harvested on the fifth day, and centrifuged to remove the cells. The use of the same basal broth supernatant helped to ensure that different systems in the study differed only in the added cells and/or CH. The cell-containing systems were added with the same cell concentration, approx 3 g/L. The CH-containing systems were added with 5% CH shortly before the foaming study. The hydrolysate added was prepared to have the same medium composition (C-source omitted) so that the hydrolysate addition had minimal effects on other broth properties.

The observations on foaming speed and foam stability are summarized in Table 1. The hydrolysate-based broth foamed readily and the foam was quite stable, significantly more so than the lactose-based broth, as described in more detail later. The presence of cells slowed down the

Table 2
Effects of Addition of Cellulose Hydrolysate and Cells of Different Culture Stages on Foam Fractionation

| | | Cells (g/L) | | | Reducing sugars (g/L) | | | FPU | | | Extracellular proteins (g/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cells | Hydrolysate (%) | Broth | Foamate | ER | Broth | Foamate | ER | Broth | Foamate | ER | Broth | Foamate | ER |
| | 0 | 0 | 0 | – | 1.2 | 1.1 | 0.9 | 0.37 | 0.46 | 1.2 | 0.16 | 0.21 | 1.3 |
| None | 5 | 0 | 0 | – | 1.6 | 1.2 | 0.8 | 0.34 | 0.7 | 2.1 | 0.14 | 0.1 | 0.7 |
| Growth | 0 | 2.3 | 1.9 | 0.8 | 1.2 | 0.9 | 0.7 | 0.37 | 0.45 | 1.2 | 0.14 | 0.1 | 0.8 |
| phase | 5 | 2 | 2.4 | 1.2 | 1.5 | 1.1 | 0.7 | 0.34 | 0.62 | 1.8 | 0.13 | 0.07 | 0.6 |
| Stationary | 0 | 2.2 | 2.1 | 1.0 | 1.1 | 0.8 | 0.8 | 0.37 | 0.42 | 1.1 | 0.15 | 0.16 | 1.1 |
| phase | 5 | 2.1 | 2.7 | 1.3 | 1.5 | 1.1 | 0.7 | 0.34 | 0.56 | 1.6 | 0.14 | 0.08 | 0.6 |

foaming speed and made the foam less stable. The addition of CH also slowed down the foaming speed in the cell-free systems but had minimal effect in the cell-containing systems.

The enrichment ratios (ER) for cells, reducing sugars, cellulase (FPU) activity, and extracellular proteins achieved are reported in Table 2. The ER for cells is defined as the ratio of cell concentration in the foamate to that in the original broth. ER for other parameters is similarly defined. The results in Table 2 indicated the following:

1. CH addition enriched cellulase in all systems. The ER was larger in the cell-free systems.
2. CH addition significantly decreased the partition of extracellular proteins that had no cellulase activity, resulting in much lower ER of proteins. Together with the increased enrichment of cellulase, the observation indicated a clear selectivity of CH toward cellulase. The complex formed between cellulase and the pertinent CH components (presumably the cellulose oligomers) out-competed the other proteins in partitioning onto the bubble/foam surface, consequently, causing the decrease in ER of proteins.
3. CH addition seemed to decrease the removal of reducing sugars, although the effect was significant only in the cell-free systems. ER of reducing sugars was smaller than 1 in all of the systems.
4. The presence of cells did not affect ER of FPU, proteins, and reducing sugars substantially, but the cells were removed by foaming. CH addition increased the extent of cell removal. Cells harvested at the two different growth stages behaved similar in the broth foaming.

The earlier observations were qualitatively reproducible in all of the subsequent foaming experiments conducted with the hardwood hydrolysate as the affinity foaming agent. Note that the hydrolysate had approx 12 g/L of reducing sugars. Thus, the 5% addition used in the above experiment corresponded to addition of approx 0.6 g/L of reducing sugars. The predominant majority of the reducing sugars in the hydrolysate were glucose (40%) and xylose (27%). Assuming that only the oligomers had the high affinity in binding cellulase and forming more hydrophobic complex for enhanced partition onto foam surface, the amount of "actual" foaming agents introduced was very low. On developing methods to produce CH with larger fractions of oligomers, the efficiency of affinity foam fractionation of cellulase may be greatly improved.

Further experiments were conducted in cell-free systems to evaluate the effects of increasing CH fractions, up to 75%, on the foam fractionation. The experiments were done with three combinations of the broth supernatant (from hydrolysate-based or glucose plus Avicel cellulose-based fermentation) and the type of CH as foaming agent (with or without autoclaving at 121°C for 15 min): System 1, nonautoclaved CH added to hydrolysate-based broth supernatant; System 2, autoclaved CH added

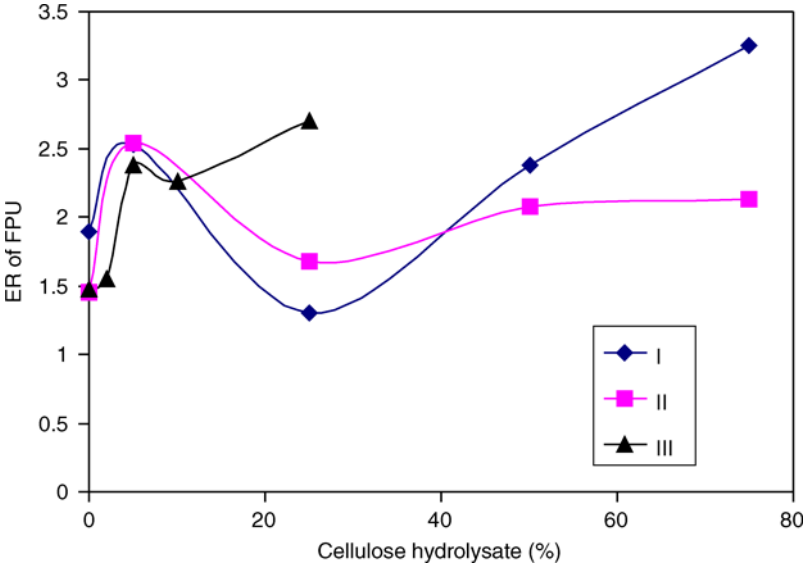


**Fig. 1.** ER of FPU at different percentages of CH for three cell-free systems: System I, hydrolysate-based broth + nonautoclaved CH; System II, hydrolysate-based broth + autoclaved CH; System III, glucose plus cellulose-based broth + autoclaved CH.

to hydrolysate-based broth supernatant; and System 3, autoclaved CH added to glucose plus cellulose-based broth supernatant. The ER of FPU achieved in the three systems, at different CH fractions, are summarized in Fig. 1. CH addition was found beneficial in all of the three combinations, giving maximal ER of 2.6–3.4. The cause for the dips at 25% CH in Systems 1 and 2 were unknown, but the trend was reproducible in both systems. Autoclaved and nonautoclaved CH behaved similar except at the very high fraction (75%).

Affinity foam fractionation by CH addition improved the purity, in addition to concentration/enrichment, of the cellulase in foamate. This is shown in Fig. 2, by the substantially higher values of E/P in foamate (enzyme-to-proteins, calculated by dividing FPU by the concentration of extracellular proteins) with increasing CH fractions for the three systems.

As described earlier, cellulase includes three groups of components: endoglucanases, exoglucanases, and β-glucosidases. It is important to evaluate the effect of CH addition on foam fractionation of individual groups of cellulase. The ER for cellulase components at different CH fractions is shown in Fig. 3 for System 2. (The profiles were essentially the same for System 1, but not measured for System 3.) Exoglucanases were the primary component enriched. Enrichment of the other two components, particularly endoglucanases, was also observed at the low CH fraction of 5%. The enrichment diminished at higher CH fractions, and for β-glucosidases, it even dropped below the level attained without CH addition. The poor enrichment in endoglucanases and/or β-glucosidases must

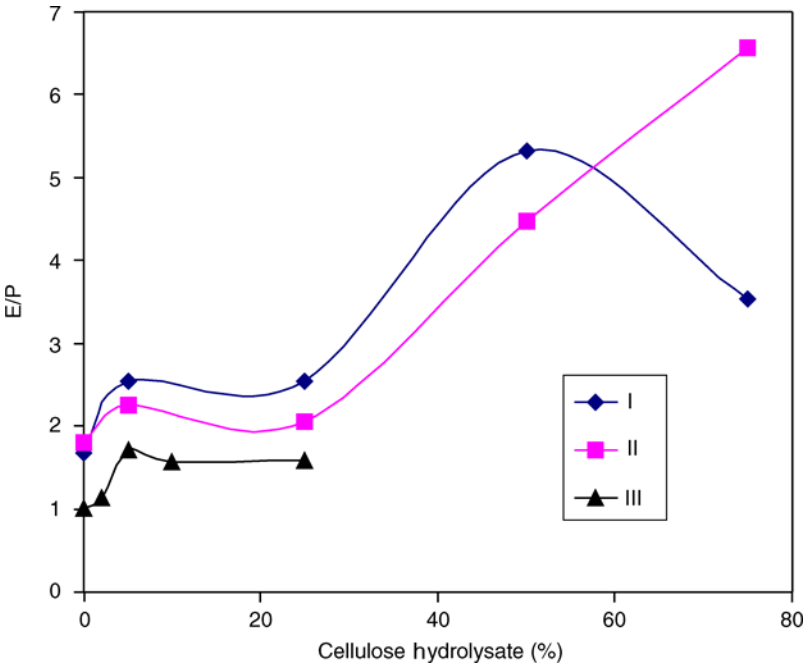


**Fig. 2.** Values of E/P at different percentages of cellulose hydrolysate for three cell-free systems (as described in Fig. 1).

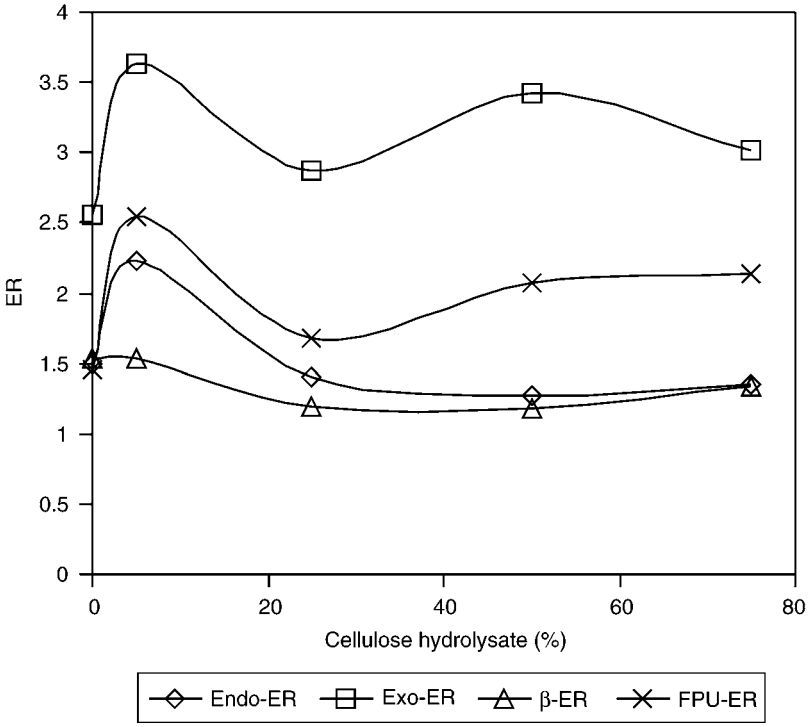


**Fig. 3.** ER of FPU and individual cellulase components, i.e., endoglucanases, exoglucanases, and β-glucosidases, at different percentages of cellulose hydrolysate for System II (as described in Fig. 1).

be responsible for the lower ER (2–2.5) of overall FPU than those (3–3.6) of exoglucanases.

The above observations are very important to the future improvement of affinity foam fractionation of cellulase. The different effects on cellulase components were probably associated with the different sizes of oligomers preferred by the different components as substrates. With the primary function of hydrolyzing cellobiose (dimer), β-glucosidases are expected to have higher affinity to smaller oligomers, which are very water soluble and tend not to partition onto the foam surface. To enhance the enrichment of β-glucosidases would require attaching the small oligomers to another hydrophobic entity so that the bound complexes would partition actively to the foam surface. On the other hand, endoglucanases function to cleave long cellulose chains. Presumably, they would have higher affinity to larger oligomers (more so than the exoglucanases, which can bind to shorter chains for their function of cleaving the chains at the end). The poor enrichment of endoglucanases observed in this work might be a result of the extremely low concentration of large oligomers present in CH, which was originally prepared to contain primarily glucose. Methods designed to obtain longer oligomers in the hydrolysate are desirable for optimizing the efficiency of the affinity foaming technology.

### *Affinity Foam Fractionation With Addition of CMC*

CMC are modified, water-soluble, long-chain cellulose analogs. The potential use of CMC for affinity foam fractionation of cellulase was therefore examined. The experiment was carried out in cell-free supernatant of the broth collected from a hydrolysate-based fermentation. To obtain higher FPU (~0.7) than that from the earlier batch cultivation (~0.3–0.4 FPU), the fermentation was supplemented with a lactose-based continuous feed after reaching the stationary phase. Three systems were compared, the broth supernatant (control), the supernatant added with 5% (v/v) of a 5 g/L CMC solution, and the supernatant added with 5% of a hardwood CH (having 16 g/L of reducing sugars). The ER of FPU, extracellular proteins, and reducing sugars are shown in Fig. 4. The CMC solution was found to perform as well as, if not better than, CH in cellulase enrichment.

CMC is available commercially in several molecular weights (MW) and degrees of substitution (DS, in introduction of the carboxylic acid group). The CMC used in the above experiment belonged to the type "7L": "7" stands for a 70% DS (i.e., on average, 70% of the glucose units have an acid group attached), and "L" stands for low MW (~90,000). To study the effects of DS and MW on the affinity foaming performance, an experiment was conducted with five systems, each added with 5% (v/v) of a specific type of CMC (10 g/L solution), 7L, 7M, 7H, 9M8, and 12M8, where M and H refer to medium and high MW (~250,000 and 700,000, respectively), and

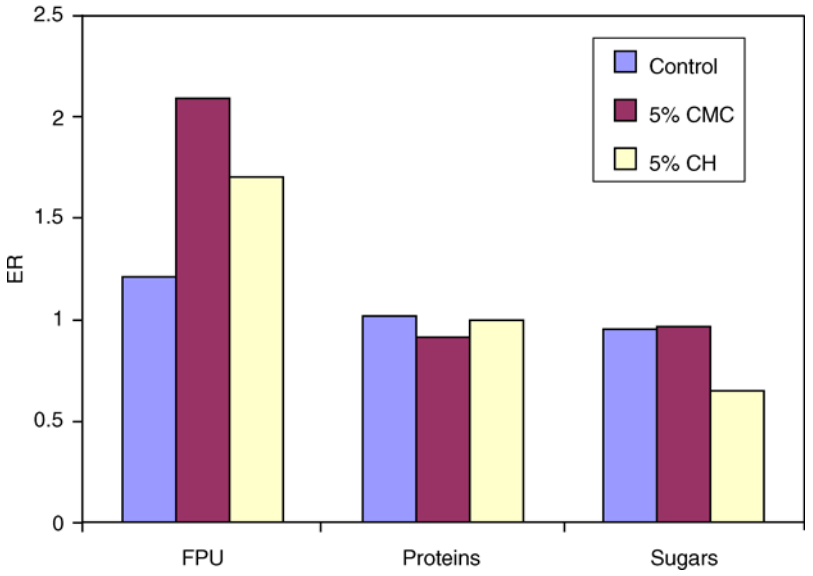


**Fig. 4.** Comparison of the effects of CMC and CH addition on foam fractionation, in terms of ER of FPU, extracellular proteins and reducing sugars. Control had no addition of CMC or CH.

9M8 and 12M8 refer to approx 90% and 120% DS (and the "8" indicates that the viscosity of a 2% solution is ~800 centipoises). The ER of FPU obtained is shown in Fig. 5. CMC with the lower DS (at 70%) performed significantly better than those of higher DS, presumably because the higher DS decreased the affinity between cellulase and the modified sugar chains. Increasing MW also had a positive effect on the cellulase enrichment.

### *Affinity Foam Fractionation With Addition of Xylan Hydrolysate*

The results of an experiment comparing the effects of xylan hydrolysate (XH) and cellulose (hardwood) hydrolysate (CH) in affinity foam fractionation of cellulase are given in Table 3. The cell-free broth supernatant was collected from the lactose-based fermentation, as described in Materials and Methods. The lactose-based broth, despite its much higher FPU (~0.9), turned out to be not very foaming. The poor foaming correlated with its much lower concentration of extracellular proteins, confirming our earlier observation that cellulase did not cause active foaming compared to certain other proteins present in the broth, and the selective separation of cellulase by foam fractionation requires the affinity foaming developed in this work.

XH had a stronger foaming ability than CH, as indicated by the substantially larger foam volumes obtained with XH than with CH in Table 3. The two hydrolysates performed similar in enrichment of reducing sugars. Compared with CH, XH had slightly lower ER for both FPU and extracellular proteins. The FPU enrichment in the lactose-based broth supernatant

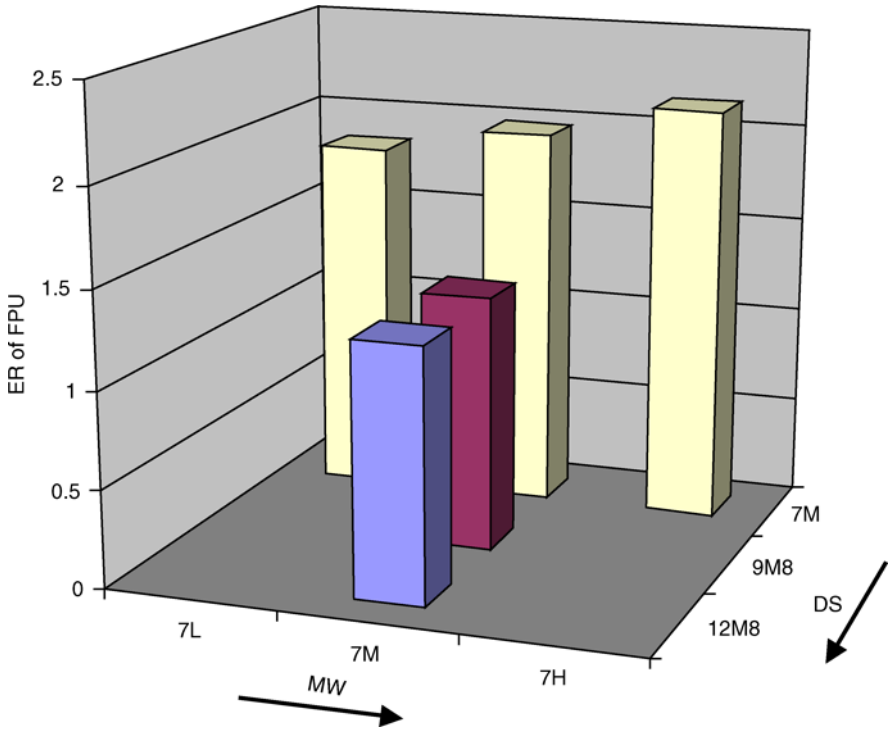


**Fig. 5.** Comparison of different types of CMC for enrichment ratios of FPU. DS refers to degree of substitution (at 70, 90, and 120%), and MW refers to molecular weight (L, low; M, medium; and H, high).

Table 3
Comparison of the Effects of XH and CH Addition on Foam Fractionation From Cell-Free, Lactose-Based Broth Supernatant, in Terms of Maximum Foam Volume, ER of FPU, Extracellular Proteins and Reducing Sugars, as well as Values of E/P (i.e., FPU/g/L of Proteins)

| | Foam volume (mL) | | ER of FPU | | ER of proteins | | ER of sugars | | E/P | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hydrolysate (%) | XH | CH | XH | CH | XH | CH | XH | CH | XH | CH |
| 25 | 20 | – | 1.3 | – | 1 | – | 1 | – | 8.7 | – |
| 50 | 20 | 10 | 1.0 | 1.7 | 0.9 | 1.4 | 0.8 | 0.9 | 8.9 | 6.3 |
| 75 | 70 | 10 | 1.5 | 1.8 | 1 | 1.5 | 1.1 | 0.9 | 18.4 | 9.2 |

Lactose-based broth did not foam well. Addition of 25% CH did not allow meaningful collection of foamate; consequently, no measurements were available for that system.

was not very high, up to approx 1.8, as compared with that in the hydrolysate-based broth supernatant, up to 3.5–4.5. However, it should be noted that there was less room for enrichment and purification in the lactose-based supernatant because it was much richer and purer in cellulase

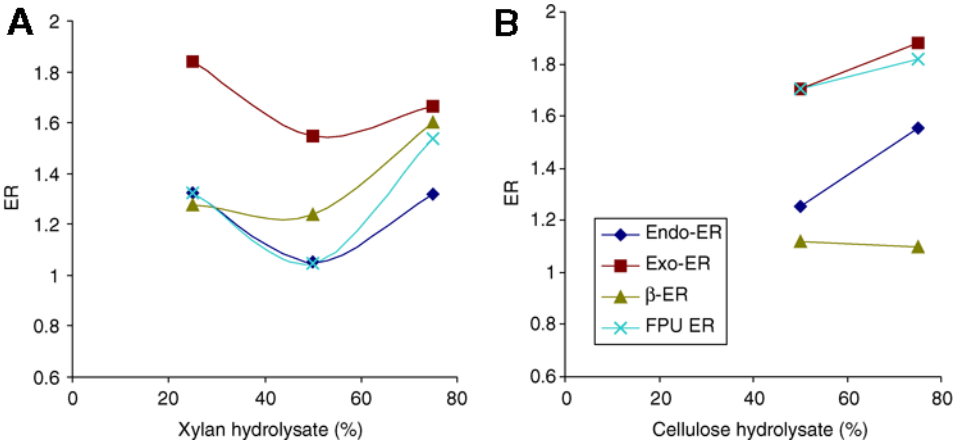


**Fig. 6.** Comparison of the effects of **(A)** XH addition and **(B)** (CH) addition on foam fractionation of FPU and individual cellulase components, i.e., endoglucanases, exoglucanases, and β-glucosidases, from cell-free, lactose-based broth supernatant.

(with E/P = 5.6) than the hydrolysate-based supernatant (with E/P = 1.1) to begin with. Although the E/P value for pure cellulase is yet to be determined, the value reached about 18 and 9 in the foamate produced with 75% XH and CH, respectively, from the lactose-based broth supernatant. Both were much higher than the E/P value (up to ~6.5) obtained with 75% CH from the hydrolysate-based broth supernatant (Fig. 2).

The effects of XH and CH, at different fractions, on foam fractionation of individual cellulase components are shown in Fig. 6. The CH-facilitated behaviors were similar in the hydrolysate-based broth supernatant (Fig. 2) and in the lactose-based broth supernatant (Fig. 6B): ER was highest for exoglucanases and lowest for β-glucosidase. XH also enriched exoglucanases the most from the lactose-based broth supernatant (Fig. 6A), but appeared to have least enrichment for endoglucanases. This last observation was rather surprising, because XH was prepared to have higher concentrations of oligomers than CH. Cellulase was nonetheless expected to have lower affinity to XH, than to CH, which might have played a role in the poorer enrichment of endoglucanases by XH.

The above results clearly demonstrated the beneficial enrichment and purification of cellulase, particularly exoglucanases, by affinity foam fractionation using CH, CMC, and XH. Conceivably, the fermentation broth can be fed into the foam fractionation columns for collecting the cellulase-enriched foamate in batch or continuous operation. The presence of cells did not seem to hamper the FPU enrichment and the cells would be present in the foamate collected. For applications stipulating cell-free formulations, a separate cell clarification step is required. Depending on the desired cellulase purity, the enriched foamate may or may not need further purification with other conventional methods. Nonetheless, the foamate volume would be

significantly smaller than the original broth volume, allowing for more economical cell clarification and/or enzyme purification. Fermentation broths are, however, notoriously complex and the intertwining factors governing the interfacial phenomena occurring in foam fractionation are far from being clearly understood or quantitatively described. More studies and further development of affinity foaming agents will prove fruitful in realizing the significant potential of the affinity foam fractionation technology.

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