Reducing the Cost of Scleroglucan Production by Use of a Condensed Corn Solubles Medium

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To reduce costs for producing the microbial exopolysaccharide scleroglucan we replaced the defined medium by one based on a low-cost byproduct of corn ethanol production. We acclimated *Sclerotium glucanicum* to grow on a condensed corn solubles (CCS) based medium, then evaluated adding the minerals used in the defined medium. We optimized the carbon to nitrogen ratio in the CCS medium, and compared the defined medium to the CCS based medium in bioreactor trials. We achieved scleroglucan levels up to 26.5 g/L in the CCS medium, compared to 13.3 g/L in the defined medium. Even accounting for CCS particulates trapped in the scleroglucan (up to 5 g/L), scleroglucan yields on the CCS were at least equal to the highest yields reported in the literature. The cost of the production medium was lowered from $3.36/kg scleroglucan for the defined medium to $0.37/kg scleroglucan in the CCS medium.

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Introduction

Scleroglucan is an extracellular polysaccharide produced by several fungal genera, including *Sclerotium, Corticium and Sclerotinia* (1, 2, 3). It is comprised of a β-(1, 3)-linear backbone of glucose, with single D-glucopyranosyl side groups attached with β-(1, 6) bonds every third or fourth glucopyranosyl molecule on the main chain (4). Scleroglucan is a neutral-charged polysaccharide (4, 3). Scleroglucan forms gels that are stable over the following conditions: 0-120^{o}C, pH 1–12.2, and salt concentrations up to 20% (5, 6, 7). Due to this wide stability, scleroglucan has great potential for use in various applications, including use in modified-release pharmaceuticals (8, 3, 4) and antitumor activity (4). However, its high cost has limited applications to markets such as chemically enhanced oil recovery (6) and as a biological response modifier. If production costs could be lowered, scleroglucan might find expanded markets in the pharmaceutical, food, and agricultural applications (9, 10, 11, 12, 13).

One approach to reducing scleroglucan production cost is to minimize capital and operating costs associated with fermentor operation. Kang et al. (1, 14) summarized the advantages and limitations of various fermentor designs, and developed a bi-staged process in an airlift reactor. Another way to reduce scleroglucan costs is to use a less expensive medium. Most studies have utilized a medium developed by Wang for cultivation of *S. glucanicum* (1, 9, 12, 13, 15, 16, 17, 18, 19). Unfortunately this medium, which contains yeast extract, various minerals, and glucose or sucrose, is too expensive for use on an industrial scale. Selbmann et al. (2) evaluated a range of raw and hydrolyzed starch materials to replace glucose in Wang's medium. They found

that partially or totally hydrolyzed corn starch could effectively replace glucose, but the medium still contained expensive ingredients such as yeast extract.

Our objective was to determine if a low-value byproduct of corn-based ethanol production would support growth and scleroglucan production by *S. glucanicum*. This byproduct, condensed corn solubles (CCS), results when ethanol is distilled from the corn fermentation broth, solids are recovered by centrifugation and evaporators concentrate the resulting supernatant. CCS contains moderate to high levels of protein, sugars, complex carbohydrates, vitamins, various micro- and macro-nutrients, as well as lysed yeast cells (20, 21, 22). Our hypothesis was that the cost of scleroglucan production could be reduced by employing CCS as the nutrient base. We further hypothesized that because of its complex nutritional profile, CCS could actually increase scleroglucan production over common lab media.

To accomplish this objective we first acclimated *S. glucanicum* to a basal CCS medium, then determined if any of the minerals in Wang's medium would boost production. Next we optimized the levels of glucose and CCS (source of nitrogen) to maximize scleroglucan production. According to Farina et al. (15, 16), Taurhesia and McNeil (23), Kwangia and Hong (24) and Rau et al. (17) the ratio of carbon to nitrogen plays a major role in optimizing biomass and scleroglucan production. We then compared the optimized CCS-based medium to Modified Wang's medium under similar conditions (aeration and agitation) in a stirred tank bioreactor. Wang and McNeil (18) and Taurhesia and McNeil (23) found that sucrose supplementation during late exponential/early stationary phase increased scleroglucan production. Therefore we subsequently evaluated fed batch fermentation in the bioreactor, supplementing 10 g/L glucose at 48 hours fermentation time.

Materials and Methods

Organism, Culture Maintenance and Media Formulations

Sclerotium glucanicum NRRL 3006 was obtained from the USDA National Center for Agricultural Utilization Research, Peoria, IL. For long-term storage, both wild-type and condensed corn solubles (CCS) acclimated strains were grown on potato dextrose agar slants, covered with sterile mineral oil, and stored at 4 ^{o}C. Cultures were routinely transferred (72-96 h) in culture media described below in section 2.1 and incubated at 28 ^{o}C in a rotary shaker (250 rpm).

Wang's medium (1, 9, 12, 13, 15, 16, 17, 18, 19) was used to initially culture the fungus and as a component in the acclimation process. Wang's medium contains (per L): 30.0 g glucose, 3.0 g $NaNO_3$, 1.0 g yeast extract, 1.0 g $K_2HPO_4.3H_2O$, and 1.0 g $MgSO_4.7H_2O$. A modified version of Wang's medium (30.0 g glucose, 1.0 g yeast extract, and 1.0 g/L $MgSO_4$-$7H_2O$ per L) was also used for culture maintenance and for comparison with the CCS-based medium in bioreactor trials. Condensed corn solubles (CCS) were obtained from two local dry-mill ethanol plants and were refrigerated until use at 4 ^{o}C. CCS from ethanol plant #1 was used for the majority of the work reported herein. CCS from ethanol plant #2 was used in one trial for comparative purposes, to determine if a strain of *S. glucanicum* which had been acclimated to CCS #1 would readily grow on CCS #2. The basal CCS medium consisted of 240 g CCS (wet weight) in 1 L deionized water, and was used for

the *S. glucanicum* acclimation trials. A medium containing 160 g/L CCS and 20 g/L glucose was also used for culture maintenance. Due to problems in separately quantifying cell biomass and CCS solids, we subsequently filtered the various CCS media through Whatman #113 filter paper to remove suspended solids. This filtered CCS medium was used for all remaining shake flask and bioreactor trials. All media formulations were adjusted to pH 4.5 with either 7.0 M H_2SO_4 or 7.0 M NaOH.

Acclimation of *S. glucanicum* to Dry-Mill CCS Medium

S. glucanicum failed to grow initially in the 240 g/L CCS medium. Therefore, we prepared mixtures of Wang's medium and the unfiltered 240 g/L CCS medium in ratios from 90:10 to 10:90 in 250 ml Erlenmeyer flasks (100 mL media). We started with 100% Wang's medium, and sequentially transferred *S. glucanicum* to media containing progressively higher levels of the CCS medium. At each transfer we used a 10% inoculum level, and then incubated at 28^{o}C in a rotary shaker (250 rpm). As growth and scleroglucan production occurred (as observed by increased viscosity), we continued to subculture into the same formulation but also transferred to hybrid media with the next three lower ratios of Wang's to CCS. When CCS levels reached greater than 50 parts, 10 g/L glucose was added to account for the decreased level of glucose available from the Wang's medium. Once *S. glucanicum* was fully acclimated to the 240 g/L CCS, 10 g/L glucose medium, we inoculated a 240 g/L CCS medium without supplemental glucose to determine if *S. glucanicum* could use the wide variety of simple and complex carbohydrates in CCS. We also evaluated growth and scleroglucan production in a filtered 240 g/L CCS, 10 g/L glucose medium.

Importance of Selected Minerals in Wang's Medium

To determine the relative importance of the minerals in Wang's medium, a series of shake flask trials were performed comparing Wang's medium, Wang's medium without minerals, and Wang's medium with the following combinations of minerals: 1.0 g $MgSO_4.7H_2O$; 1.0 g $MgSO_4.7H_2O$ + 1.0 g $K_2HPO_4.3H_2O$; 1.0 g $MgSO_4.7H_2O$ + 1.0 g $NaNO_3$; 1.0 g $K_2HPO_4.3H_2O$; 1.0 g $K_2HPO_4.3H_2O$ + 1.0 g $NaNO_3$. We then conducted a similar trial to see if these minerals would improve performance of the filtered CCS medium. Here we added the following combinations to the filtered 240 g/L CCS, 10 g/L glucose medium: no additional nutrients, 1.0 g/L $MgSO_4.7H_2O$, 1.0 g/L $K_2HPO_4.3H_2O$, and 1.0 g/L of both $MgSO_4.7H_2O$ and $K_2HPO_4.3H_2O$. Yeast extract and $NaNO_3$ were not added due to the presence of sufficient nitrogen in the CCS, and the apparent inhibitory effects of $NaNO_3$ that we observed in the prior trials. All these trials were conducted in 1 L Erlenmeyer flasks containing 500 mL of medium adjusted to pH 4.5 with NaOH or H_2SO_4. The larger volumes were required so that 50 mL samples could be withdrawn periodically for scleroglucan quantification, along with HPLC and pH analysis. Flasks were incubated at 28^{o}C in a rotary shaker (250 rpm). All trials were performed in triplicate.

Determining the Optimal C:N Ratio for the Basal Dry-Mill CCS Medium

Various investigators (12, 17, 24) have noted that carbon to nitrogen ratio affects scleroglucan production, hence we conducted trials to identify optimal CCS and glucose levels. These trials compared the following matrix: 0, 10, 15, 20, 30, and 40 g/L glucose in 80, 120, 160, 240, and 360 g/L CCS (pre-filtered wet weights). Trials were again performed in 1 L

Erlenmeyer flasks containing 500 mL media, adjusted to pH 4.5 with NaOH or H_2SO_4. Flasks were inoculated with 5% inoculum of a 48 - 72 h culture of *S. glucanicum*, and were incubated for up to 6 days at 28^{o}C and 250 rpm agitation. All trials were performed in triplicate.

Bioreactor Trials

Based on prior results, we prepared a simplified version of Wang's medium containing 30.0 g glucose, 1.0 g yeast extract, and 1.0 g $MgSO_4.7H_2O$ (omitting the 3.0 g $NaNO_3$ and 1.0 g $K_2HPO_4.3H_2O$). We compared this modified Wang's medium to the optimal filtered CCS medium (160 g/L pre-filtered wet weight CCS, 20 g/L glucose) in a 5 L BioFlow III reactor (3 L media) operated at 28^{o}C, 350 rpm, and an aeration rate of 1 volume/volume/min. Trials were inoculated with a 5% inoculum of a 48 h culture grown in the corresponding medium to the trial. Cultures were incubated for 144 h, and samples were taken at 12-h intervals and analyzed as described in the shake flask trials of above section. Fed-batch trials were also performed, as some investigators had observed catabolite repression at sugar levels in excess of 40 g/L (13, 17, 18, 23). Conditions were similar to that described above in section 2.5, but at 48 h the glucose concentration was increased by 10 g/L by adding an appropriate volume of 300 g/L sterile glucose solution.

Analytical Methods

Sample pH was measured using an Accumet 950 pH/ion meter (Fischer Scientific). Samples were analyzed by HPLC for sugars, organic acids, ethanol, and glycerol. Samples were filtered through a non-sterile 0.2 µm nylon membrane filter to remove cells and solids, placed in autosampler vials, and frozen until analysis. We used a Waters HPLC system with an Aminex®HPX-87H column operated at 58^{o}C with a 4 mM H_2SO_4 helium-degassed mobile phase at a flow rate of 0.6 mL/min. Peaks were detected using a refractive index detector.

Scleroglucan and cell biomass were recovered from culture broth using a standard protocol (1, 18, 19, 23, 25). Culture broth (50 mL) was centrifuged at 10,000 rpm for 20 min to separate supernatant (containing scleroglucan) from the cell pellet. The pellet was then washed in an equal volume of water and recentrifuged to remove any adhering gum. The cell pellet was dried to a constant weight at 55^{o}C. The cell-free supernatant fractions were then mixed with two volumes of ethanol to precipitate scleroglucan. The precipitate was recovered by vacuum filtration and dried to a constant weight at 55^{o}C.

Results and Discussion

Acclimation of *S. glucanicum* to Dry-Mill CCS Medium

To reduce scleroglucan production costs, the first objective of this project was to acclimate *Sclerotium glucanicum* to the lower cost alternative production medium, condensed corn solubles (CCS). This was necessary since initial attempts to grow *S. glucanicum* in the 100% CCS medium were unsuccessful. Thus, acclimation was performed using hybrid media containing various levels of CCS and Wang's medium. Cultures were monitored daily for growth and scleroglucan production, and were plated prior to each transfer to ensure culture purity. When cultures exhibited an acceptable level of growth and sceleroglucan production, they were transferred to media with lower levels of Wang's medium. To account for the gradual reduction in sugar (contributed by Wang's medium), when CCS levels exceeded 50% of the mixture, a supplement of 10 g/L

glucose was added. This process was continued for approximately 10 weeks, until *S. glucanicum* was able to grow on a 100% CCS based medium. Then the culture was successfully grown on a 100% CCS medium without glucose supplementation, demonstrating that the carbohydrates in CCS were sufficient to support growth.

Table 1. Analysis of Condensed Corn Solubles (CCS) from Two Dry Mill Ethanol Plants[a]

Component	Ethanol Plant #1[b]	Ethanol Plant #2[c]
Moisture	76.0	65.1
Dry Matter	24.0	34.9
Crude Protein	27.5	13.7
Crude Fat	15.7	16.2
Ash	7.4	9.5
Nitrogen Free Extract	45.4	59.9
Calcium	0.08	0.05
Phosphorus	1.21	1.54
Magnesium	0.58	0.69
Potassium	1.81	2.29
Sodium	0.19	0.56

[a] Composition on a dry matter basis (%, w/w).
[b] CCS used for initial acclimation of *Sclerotium glucanicum*, evaluating mineral supplementation, determining optimal C:N ratio, and bioreactor trials.
[c] CCS used to evaluate performance of acclimated *Sclerotium glucanicum* strain on CCS from a different dry mill ethanol plant.

We subsequently tested this acclimated strain in 100% CCS media prepared from CCS obtained from a second dry mill corn ethanol plant. This was done because CCS composition varies from plant to plant, even when they use identical processes. Table 1 shows the analysis of the CCS from these two dry mill ethanol plants; the primary difference being a lower protein and higher nitrogen free extract content in CCS from ethanol plant #2. The acclimated strain readily grew and produced scleroglucan in CCS from this second dry-mill facility. This was an important finding, because microbes used for industrial processes must be adaptable to minor variations in the composition of the cultivation medium, without significant changes to biomass and product production (26).

Importance of Selected Minerals in Wang's Medium

We next sought to determine the relative importance of the minerals in Wang's medium ($MgSO_4$, K_2HPO_4, and $NaNO_3$) by conducting trials with various combinations of these minerals. Wang's medium and Wang's medium without any minerals were used as controls. Results in Figure 1 show that while cell biomass levels ranged between 5.2 - 5.8 g/L in both Wang's medium and Wang's medium without minerals, the latter had significantly higher scleroglucan levels (5.3 g/L) compared to Wang's medium (1.8 g/L). We also observed low scleroglucan (and cell biomass) levels in treatments containing $NaNO_3$, thus indicating that this mineral was inhibitory to *S. glucanicum*. This finding corresponds with the results of previous investigators who noted that nitrogen limitation stimulated scleroglucan production (12, 13, 15, 16, 19, 24, 25). On the other hand, treatments containing $MgSO_4$ or $MgSO_4$ + K_2HPO_4 resulted in significantly higher levels of scleroglucan than the controls. However when K_2HPO_4 was added alone, scleroglucan and biomass both fell back to levels similar to those observed with trials with no minerals. This indicated that $MgSO_4$ was the stimulatory mineral and that K_2HPO_4 had no effect. Prior studies noted that phosphorus limitation enhanced scleroglucan production, and so it was not surprising that K_2HPO_4 supplementation had no effect (12, 13, 15, 16, 19, 24, 25). As a result of these trials, and due to the fact that biomass and scleroglucan concentrations were similar to those given in

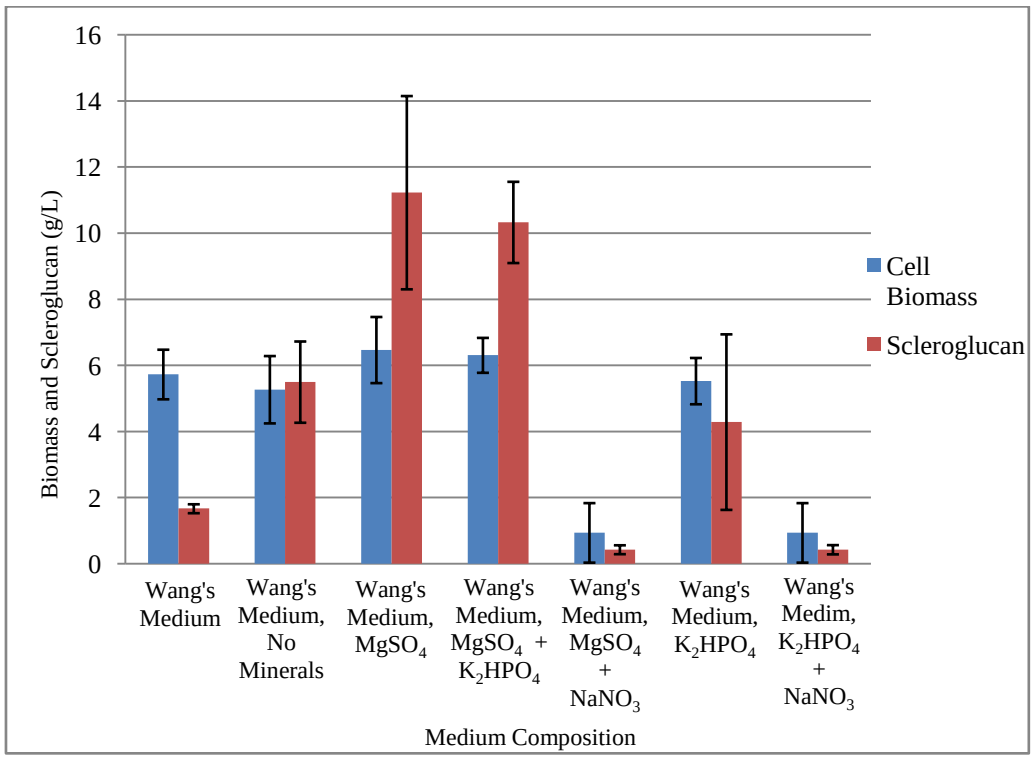

Figure 1. Effect of Selected Minerals on Biomass and Scleroglucan Production in Wang's Medium[a].
[a] Composition at 120 h. Error bars represent the standard deviation of three replications.

the literature, the Modified Wang's medium (glucose, yeast extract, and $MgSO_4$) was used as the defined medium for subsequent trials.

Due to the above findings, we next evaluated the effects of adding various combinations of $MgSO_4$ and K_2HPO_4 to the filtered CCS medium to see if scleroglucan and/or biomass productivity would increase. The data (Figure 2) indicated that neither mineral, either alone or in combination, appeared to have any significant effect on either scleroglucan or biomass production. This was likely due to the excess of these minerals in the filtered CCS-based medium.

Determining the Optimal C:N Ratio for the Basal Dry-Mill CCS Medium

The ideal C:N ratio is a major factor in scleroglucan production (12, 17, 24, 25). Since it is difficult to calculate the biologically available carbon and nitrogen in CCS for *S. glucanicum*, we conducted a series of shake flask trials to determine the optimal levels of glucose and CCS. Our goal was to maximize scleroglucan titer, productivity, and yield, while achieving high fermentation efficiency. In these trials we added 0, 10, 15, 20, 30, or 40 g/L glucose to 80, 120, 160, 240, or 360 g/L CCS (pre-filtered wet weight). Three replications were conducted and the results averaged. Figures 3-6 show the scleroglucan concentration, productivity, yield, and fermentation efficiency for these trials. Because CCS contains ~ 3 g/L glucose, we used the total glucose concentration to calculate both scleroglucan yield and fermentation efficiency.

Scleroglucan concentration, productivity, and yield were limited at low and high

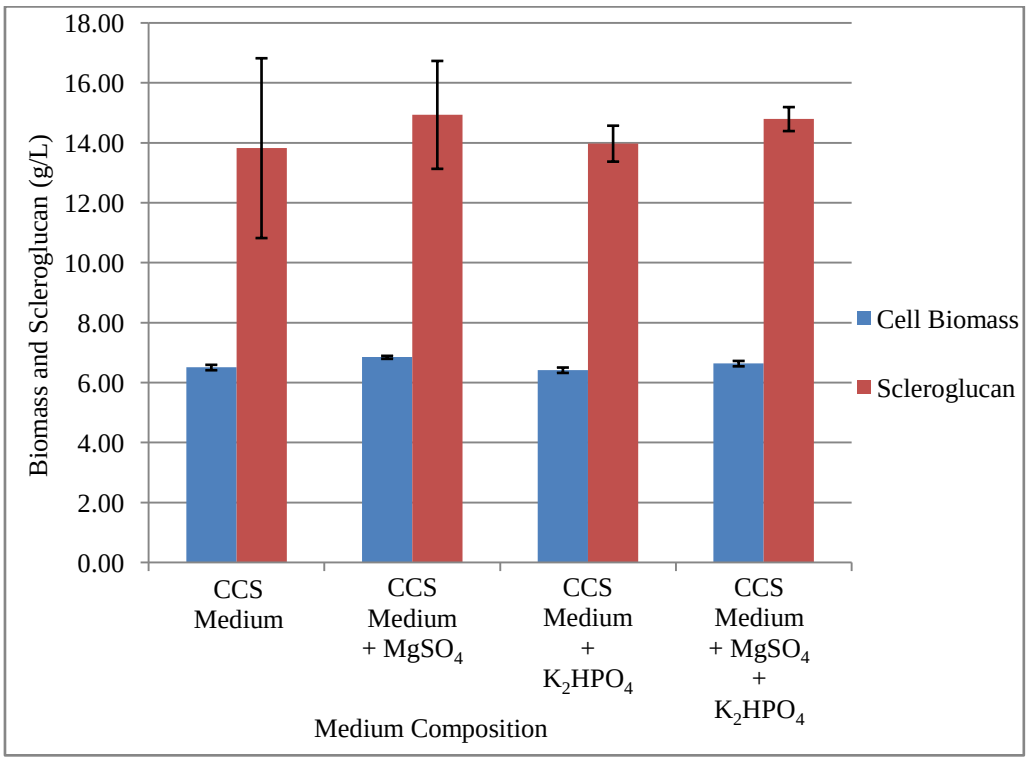

Figure 2. Effect of Selected Minerals on Biomass and Scleroglucan Production in Filtered CCS Medium[a].

[a] Composition at 120 h. Error bars represent the standard deviation of three replications. CCS medium contains 240 g/L CCS and 10 g/L glucose, and then filtered.

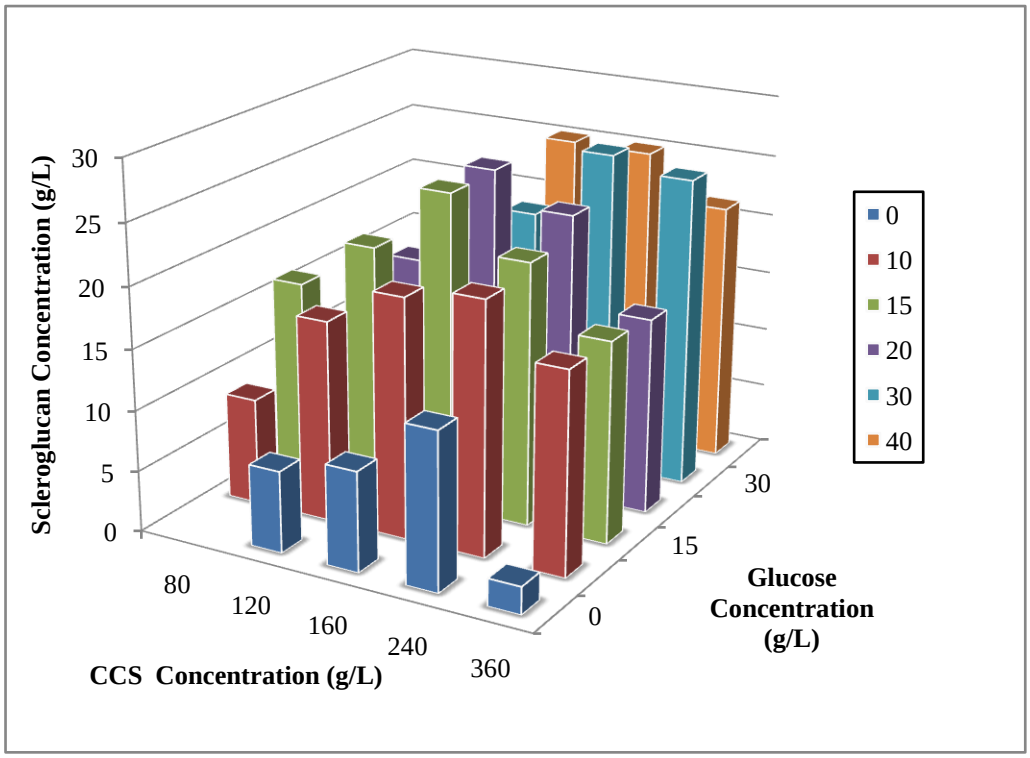

Figure 3. Scleroglucan Concentration as a Function of CCS and Glucose Concentration.

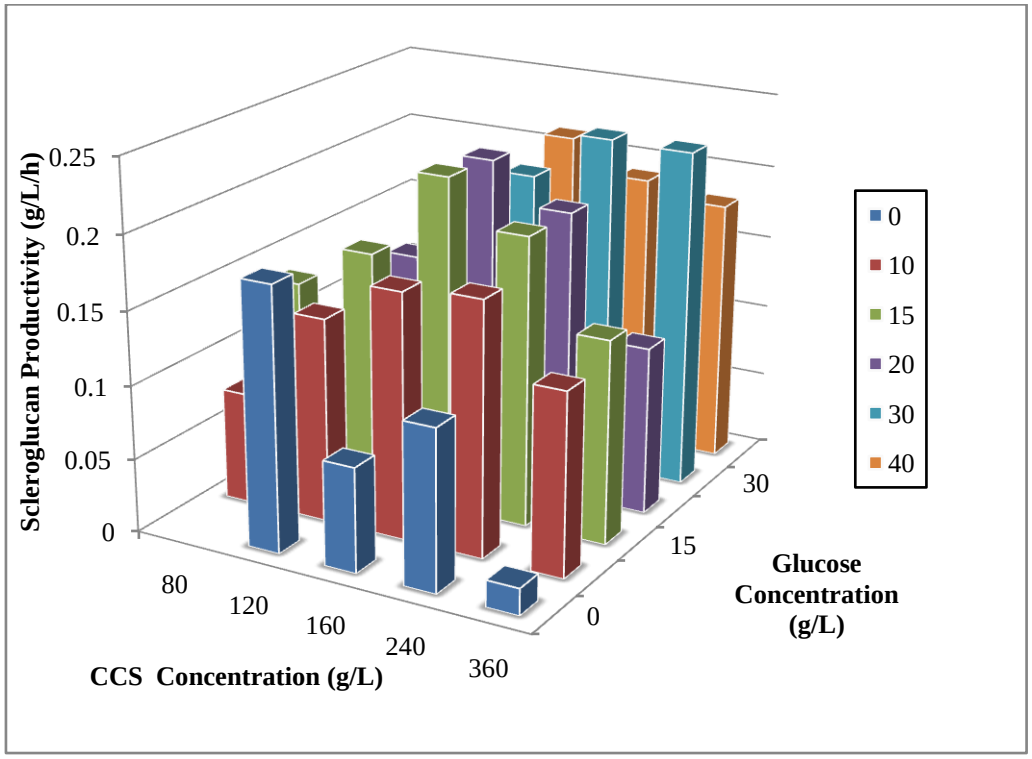

Figure 4. Scleroglucan Productivity as a Function of CCS and Glucose Concentration.

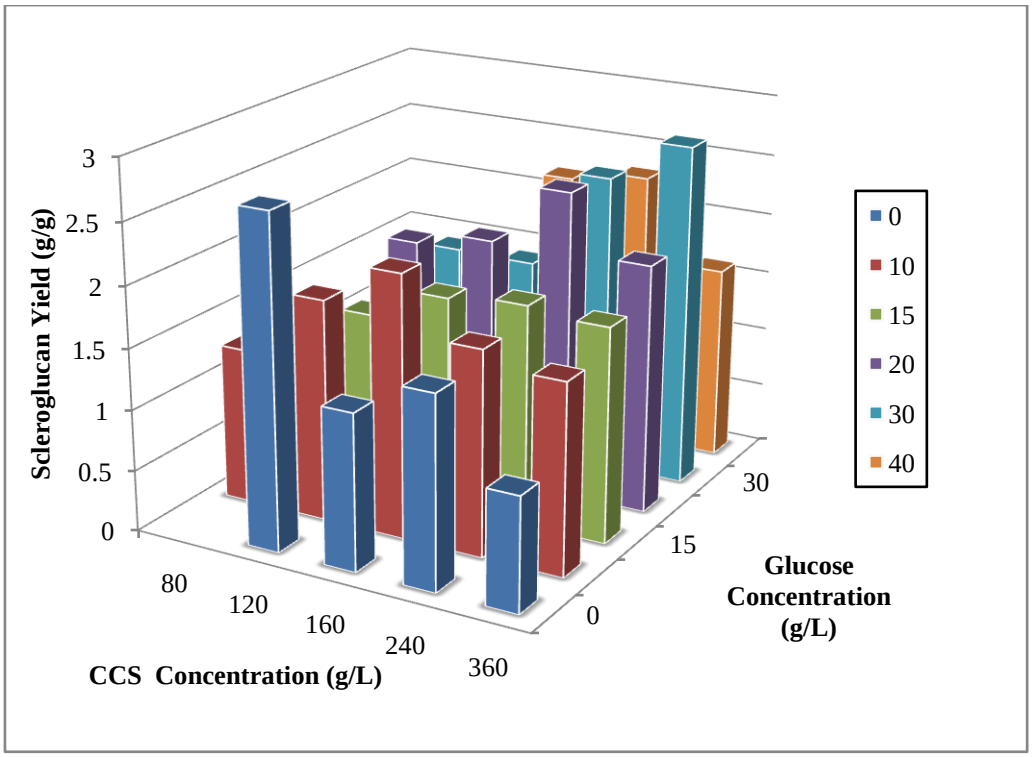

Figure 5. Scleroglucan Yield as a Function of CCS and Glucose Concentration.

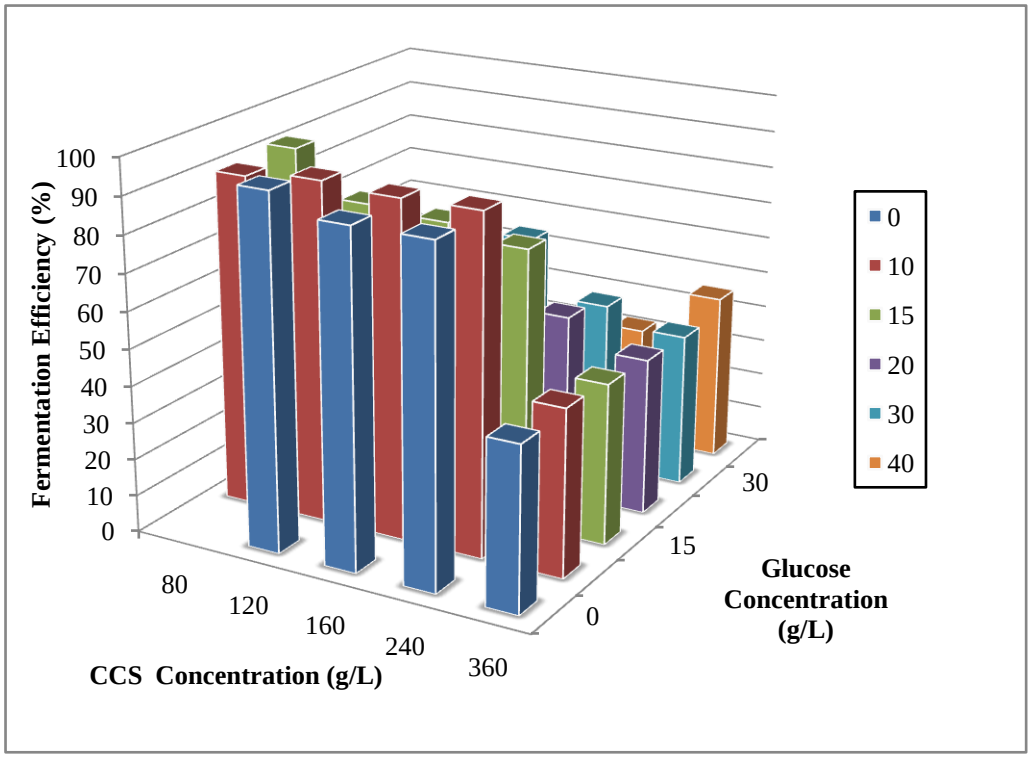

Figure 6. Fermentation Efficiency as a Function of CCS and Glucose Concentration.

concentrations of glucose and CCS. When only 0-10 g/L glucose was added, most of the glucose was apparently used for growth, leaving little for scleroglucan production. High glucose levels (40 g/L) appeared to slightly repress biomass and scleroglucan production, perhaps as the result of catabolite repression, as noted previously (13, 17, 18, 23). Low CCS concentrations (less than 160 g/L) likely do not provide enough nitrogen and/or other nutrients needed for synthesis of cell biomass and enzymes critical for scleroglucan production. High CCS levels (360 g/L) repressed scleroglucan production, either by providing too much nitrogen relative to carbon and/or exceeding the concentration of some inhibitory component in the CCS (20, 21). The fact that fermentation efficiency remained low in all of the trials using 360 g/L CCS lends support to the latter explanation.

For each of the scleroglucan production parameters (concentration, productivity, and yield) we determined the best glucose : CCS ratios at the $p<0.05$ level. Comparing the data across these three parameters, we identified the best formulations as: 30 g/L glucose : 240 g/L CCS, 15 g/L glucose : 160 g/L CCS, and 20 g/L glucose : 160 g/L CCS. Thus, the optimal C : N ratio was approximately 1 g/L glucose : 8 g/L CCS. However, at glucose levels of 15 g/L and above, fermentation efficiencies were reduced. To improve this fermentation efficiency and boost scleroglucan concentrations, bioreactor trials were attempted so that aeration and agitation conditions could be optimized.

Bioreactor Trials with Modified Wang's Medium

Modified Wang's medium (glucose, yeast extract, and $MgSO_4$) was evaluated in a

triplicate set of bioreactor trials to establish baseline levels for cell biomass and scleroglucan production. Trials were performed in a 5 L bioreactor with 3 L of media for 144 h, at 28°C, 350 rpm and 1 volume/volume/minute aeration rate. Glucose levels dropped to ~4 g/L by 82 h, with a corresponding increase in cell biomass and scleroglucan. Cell biomass reached ~6 g/L by 82 h, and slowly increased to 7.3 g/L by the end of the trials. Scleroglucan levels reached ~13.3 g/L by 82 h. Table 2 summarizes key parameters of these trials, compared to prior shake flask trials in this medium. While cell biomass (6.5 g/L) and scleroglucan (11.3 g/L) levels were only slightly lower in shake flask trials, fermentation efficiency was greatly improved in the bioreactor trials (87.9%). This can be accounted for by the slight increases in biomass and scleroglucan production, and perhaps generation of alternative by-products. For example, ~2.0 g/L oxalic acid was detected via HPLC in bioreactor trials (data not shown).

Bioreactor Trials with CCS Based Medium

The CCS medium, 20 g/L glucose and 160 g/L CCS (pre-filtered wet weight), was chosen for the bioreactor trials because in the shake flask trials it produced the highest scleroglucan levels at the highest productivity. Its main limitation was a low fermentation efficiency (62.8%, Figure 6), but we hypothesized that under better aeration/agitation conditions in the bioreactor, glucose conversion to biomass and scleroglucan would be boosted. Otherwise, conditions were identical to those used previously, and Table 2 summarizes key parameters of these trials.

Glucose was used at a similar rate to the trials in Modified Wang's medium, but was exhausted by 96 h, providing the desired boost to fermentation efficiency. This was accompanied by a corresponding increase in cell biomass (~15 g/L) and scleroglucan (~25 g/L) by 72 h. The maximum scleroglucan level of 26.5 g/L was similar to that obtained in shake flask trials using the filtered CCS medium (26.1 g/L) but cell biomass concentrations of 15 g/L were significantly higher (6.2 g/L). These results would seem to confirm that poor aeration in the prior shake flask trials limited growth. Because glucose was depleted by this increased cell growth, scleroglucan levels did not significantly increase. Nevertheless, scleroglucan productivity was improved in the bioreactor trials.

Table 2 also compares the Modified Wang's medium to the filtered CCS-based medium (20 g/L glucose, 160 g/L CCS) under bioreactor conditions. Cell biomass and scleroglucan level were twice as high in the filtered CCS medium compared to the Modified Wang's medium. Scleroglucan productivity, yield and fermentation efficiency were also higher in the CCS based medium. The yield reached levels over 1g/g in the CCS medium (shake flask and bioreactor) as the result of additional unquantifiable carbon sources (maltose, glycerol and other more complex carbohydrates) available to *S. glucanicum* in this medium.

Overall, the CCS medium appears to be better suited for scleroglucan production than the Modified Wang's medium. There are several possible reasons for this. First, scleroglucan concentrations in CCS medium may be over-estimated. If CCS particulates are not removed during medium preparation (filtering) or are trapped in the scleroglucan during centrifugal recovery of scleroglucan, the scleroglucan levels may be overestimated by perhaps as much as 5 g/L. A second reason for increased scleroglucan

Table 2. Comparison of Media and Culture Conditions on Scleroglucan Production[a].

Parameter	Shake Flask		Bioreactor		
	Modified Wang's[b]	Filtered CCS[c]	Modified Wang's[b]	Filtered CCS[c]	Fed- Batch Filtered CCS[d]
Scleroglucan (g/L)	11.3 +/-1.5	26.1 +/-2.2	13.3 +/-0.4	26.5 +/-1.0	25.5 +/-0.8
Cell Biomass (g/L)	6.5 +/-0.9	6.2 +/-1.0	7.2 +/-1.8	15.0 +/-1.6	14.5 +/-1.2
Scleroglucan Productivity (g/L/h)[e]	0.10 +/-0.02	0.22 +/-0.02	0.15 +/-.01	0.46 +/-.01	0.31 +/-.04
Scleroglucan Yield (g/g glucose consumed)	0.79 +/-0.11	2.02 +/-0.28	0.93 +/-0.13	1.13 +/-0.19	0.76 +/-0.16
Fermentation Efficiency (%)	47.3% +/-1.9	62.8% +/-6.3	87.9% +/-3.2	98.6% +/-0.8	80.3% +/-1.8

[a] Represents the standard deviation between 3 replications.

[b] Modified Wang's Medium contains 30 g/L glucose, 1 g/L yeast extract, and 1 g/L $MgSO_4$.

[c] Filtered CCS Medium contains 20 g/L glucose and 160 g/L CCS (wet weight).

[d] In Fed-batch trials 10 g/L glucose was added at 48 h.

[e] Calculated at initiation of the stationary phase.

yield in the CCS medium is potential use of complex carbon sources and nutrients in CCS. We observed a slight decrease in the levels of maltose and glycerol after glucose was exhausted (data not shown). A third potential reason is better buffering capacity in the CCS medium. The pH in the CCS medium fell from 4.5 to 3.5 during both shake flask and bioreactor trials, while it fell to 3.0 in the Modified Wang's medium. This would expose scleroglucan producing enzymes to less than optimal pH sooner in the Modified Wang's medium (19, 27, 28).

Fed-Batch Bioreactor Trials with CCS Based Medium

Rau et al. (17), Tauhesia and McNeil (23), and Wang and McNeil (18) all observed an increase in scleroglucan concentrations by 6 –7 g/L when sucrose was supplemented during late exponential/early stationary phase. This fed-batch mode prevented catabolite repression of *S. glucanicum,* that can occur at even moderate sugar levels (40 g/L). Based on the results of batch fermentation using the filtered CCS medium we estimated that 48 h would be the optimal time to supplement with 10 g/L glucose. Results from fed-batch trials are also shown in Table 2. The final scleroglucan and cell biomass concentrations (25.5 g/L and 14.5 g/L) were not significantly higher than in the corresponding batch fermentation. However, fermentation efficiency was lower (80.3%), due to the residual 6 g/L glucose. We had expected that the additional glucose would boost scleroglucan production, but did not achieve this. The maximum glucose level in this trial was 33 g/L, which is below the level reported to cause catabolite repression (13, 17, 18, 28). Taurhesia and McNeil (23) noted that timing of sucrose supplementation was critical in boosting yield.

Cost Analysis of Defined and CCS-Based Media

The primary goal of this study was to lower the cost of producing scleroglucan from *S.*

Table 3. Cost Analysis of CCS-based Medium versus the Defined Medium (Modified Wang's Medium).

	Modified Wang's Medium		CCS -Based Medium	
Component	**Amount (g/L)**	**Cost ($/Kg Scleroglucan)**	**Amount (g/L)**	**Cost ($/Kg Scleroglucan)**
Glucose	**30**	**$ 0.50**	**20**	**$ 0.17**
$MgSO_4$	**1.00**	**$ 2.01**	**--**	**--**
Yeast Extract	**1.00**	**$ 0.85**	**--**	**--**
CCS	**--**	**--**	**160**	**$ 0.20**
Total Medium Cost ($/Kg Scleroglucan)		**$ 3.36**	**--**	**$ 0.37**

glucanicum. Table 3 shows the cost per kg for producing scleroglucan from the Modified Wang's medium compared to the filtered CCS-based medium. As is shown, by replacing $MgSO_4$ and yeast extract with CCS, we were able to reduce medium costs for scleroglucan production from $3.37 in the defined medium to $0.36 in the CCS-based formulation. This has the potential to make scleroglucan production much more economically competitive using a CCS medium.

Conclusions

S. glucanicum was acclimated to produce scleroglucan on an inexpensive byproduct of the corn ethanol industry, condensed corn solubles (CCS). The optimized medium contained 20 g/L glucose and 160 g/L CCS (filtered). Bioreactor trials using this CCS medium achieved a cell biomass of 15 g/L, scleroglucan titer of 26.5 g/L, yield of 1.13 g/g, productivity of 0.46 g/L/h, and fermentation efficiency of 98.6%. These were much higher than for the defined medium (Wang's), while the cost of the median was reduced from $3.36/kg scleroglucan to $0.37/kg scleroglucan. However, improvements are needed for scleroglucan to become competitive with current industrial gums.

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