

Development of a low-cost medium for producing gellan from *Sphingomonas paucimobius*

Andrea Vanderhoff, William R. Gibbons*, Nichole Bauer, Thomas P. West

Biology & Microbiology Department, South Dakota State University, Brookings, SD 57007, USA

This study developed an inexpensive medium for producing the exopolysaccharide gellan by growing *Sphingomonas paucimobilis* on the ethanol processing co-product condensed corn solubles (CCS). We acclimated *S. paucimobilis* to grow on a medium composed of 58.6 g/L (dry basis) CCS and 30 g/L glucose by progressive transfers in media composed of blends of the defined medium and the CCS medium. Then we varied the initial glucose concentration (0-300 g/L) in the CCS medium, and determined that 30 g/L glucose was optimal for cell population (6.0×10^{10} CFU/ml), gellan concentration (12.5 g/L), productivity (0.09 g/L/h), and yield (0.80 g/g). Benchtop bioreactor trials resulted in even higher cell numbers (4.4×10^{10} CFU/ml), gellan titers (13.4 g/L), productivity (0.18 g/L/h), yield (0.57 g gellan/g glucose) and glucose utilization efficiency (90%). These levels were generally higher than those obtained in the defined medium, and therefore the glucose-supplemented CCS medium is a viable alternative for commercial production of gellan.

* Corresponding author: South Dakota State University, Dairy Microbiology 215, Brookings, SD 57007, USA. Tel. (605)688-5499; Fax (605)688-6677; E-mail: William.Gibbons@sdstate.edu.

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Introduction

The anionic polysaccharide gum gellan is produced extracellularly by the bacterium *Sphingomonas paucimobilis* ATCC 31461 [1, 2]. Gellan is composed of glucose, glucuronic acid and rhamnose linked in a molar ratio of 2:1:1 in a linear tetrasaccharide repeat [3]. The degree to which gellan is acetylated affects its ability to form a gel [4]. Monovalent and divalent cations also influence the rate of gelation of the polysaccharide [5, 6, 7, 8, 9]. Gellan is able to withstand four autoclaving cycles, is resistant to a wide range of pH changes and is tolerant of high salt concentrations. Because of these properties, this polysaccharide has applications as a thickening agent or as an adhesive in foods and as an agar substitute [5, 10, 11, 12, 13]. Gellan has potential for use as a low-cost

substitute for guar in soil erosion control products like Soil Guard™ [14, 15].

S. paucimobilis has the ability to convert six carbon mono- and disaccharides into gellan. A number of carbon and nitrogen sources have been shown to support gellan production [1, 2, 16, 17, 18, 19, 20, 21, 22]. Yeast extract supplementation of the culture medium has been shown to affect gellan production [23]. Other physiological factors such as pH and incubation temperature have also been shown to influence polysaccharide production [17, 24, 25].

The medium typically used for gellan production by *S. paucimobilis* ATCC 31461 (hereafter referred to Manna's medium) contains yeast extract, ammonium salts,

potassium phosphate, magnesium sulfate and trace elements [1, 9]. Although these ingredients support a high level of gellan production, their high cost would make the gum prohibitively expensive for use in bulk applications such as Soil Guard™. Clearly, a need exists to develop a lower-cost production medium for bacterial gellan synthesis.

West and Strohfus showed that co products from the wet-milling of corn, namely corn steep solids and corn steep liquor, could be used as nitrogen sources to support bacterial gellan production [20]. Fiahlo et al. used cheese whey was used as a carbon source for gellan production, since it contains high concentrations of lactose (5-30 g/L) [21]. However, gellan productivity was limited due to the low nutritional value of the cheese whey. Recently, a molasses-based medium for gellan production by *S. paucimobilis* ATCC 31461 was developed, but it included casamino acids which would make the medium less economical for commercial use [26].

Condensed corn solubles (CCS), a low-value co-product from dry-mill corn ethanol production, can be used as an inexpensive nitrogen source for a variety of microbes. [27, 28, 29, 30]. In the dry mill process, whole com is finely milled, mixed with water, enzymatically hydrolyzed to convert starch to glucose and subsequently fermented to convert glucose to ethanol. Following distillation, the larger com particulates (fiber and protein) are recovered by centrifugation as wet distillers' grains. The resultant supernatant (aka. thin stillage) is concentrated as CCS by evaporation. Depending upon the individual operation, the CCS can range from 25-50% dry matter and contains proteins, sugars, yeast cells, and lactic acid [31]. Thus in this investigation, we evaluated use of

CCS from corn dry-milling as a possible substitute for the ammonium salts, peptone and yeast extract in the medium typically used for gellan production. The optimal concentration of glucose as a carbon source was determined in the CCS-based medium, and subsequently evaluated gellan production using *S. paucimobilis* ATCC 31461 in 5L bioreactor trials.

Materials and Methods

Strain and culture maintenance

Shingomonas paucimobilis ATCC 31461 was used. Because culture viability on liquid or solid media under refrigeration is unacceptably low [32], we continually subcultured onto fresh media at 48-72 h intervals for short-term storage. Initially this was done using 100 ml of tryptic soy broth (TSB) in 250 ml Erlenmeyer flasks. Once *S. paucimobilis* had been acclimated to the CCS medium, subculturing was performed in 100 ml of 100% CCS medium supplemented with 30 g/L glucose. Flasks were incubated on a rotary shaker (250 rpm) at 30°C. For long term storage tryptic soy broth-grown cells of *S. paucimobilis* were recovered by centrifugation, resuspended in a 100 g/L sucrose solution, lyophilized, and stored at -4°C.

Media

The defined medium described by Manna et al. [9] was used as the control. This medium contains 0.5 g/L K_2HPO_4 , 0.5 g/L yeast extract 0.1 g/L NH_4NO_3 , 30 g/L glucose and 1 ml of a salt solution per liter. The salt solution contains 1.8 g/L $MnCl_2 \cdot 4H_2O$, 2.49 g/L $FeSO_4 \cdot 7H_2O$, 0.285 g/L H_3BO_3 , 0.027 g/L $CuCl_2$, 0.021 g/L $ZnCl_2$, 0.074 g/L $CoCl_2 \cdot 2H_2O$, 0.023 g/L $MgMoO_4 \cdot 2H_2O$ and 2.1 g/L sodium tartrate. Table 1 provides the source and purity for these chemicals. The pH was adjusted to 6.5 before use.

Table 1. Source and Purity of Chemicals used in Preparing Media

| Name | Company | Purity (%) |
|---------------------------------------|-------------------|------------|
| K ₂ HPO ₄ | Fisher Scientific | 99+ |
| Yeast Extract | Fisher Scientific | NA |
| NH ₄ NO ₃ | Sigma | 98 |
| Glucose | Fisher Scientific | 99.5% |
| MnCl ₂ ·4H ₂ O | Fisher Scientific | 99+ |
| FeSO ₄ ·7H ₂ O | Fisher Scientific | 99+ |
| H ₃ BO ₃ | Fisher Scientific | 99.99 |
| CuCl ₂ | Fisher Scientific | 99 |
| ZnCl ₂ | Fisher Scientific | >97 |
| CoCl ₂ ·2H ₂ O | Fisher Scientific | >95 |
| MgMoO ₄ ·2H ₂ O | Fisher Scientific | 99 |
| Sodium Tartrate | Fisher Scientific | 99 |

The basal CCS medium contained 240 g of CCS (equivalent to 58.6 g dry basis) in 1 L of deionized water. The pH was adjusted to 6.5, then the medium was filtered through Whatman #113 filter paper to remove suspended solids. This was necessary so that the CCS solids would not interfere with gellan and biomass quantification. This concentration was selected since it approximates the solids concentration of thin stillage before it is evaporated to form CCS. The dry mill CCS was obtained from a regional ethanol plant, and was refrigerated until use. It contained 24.0% dry matter, 27.5% crude protein, 15.7% crude fat, 1.8% potassium, 1.2% phosphorus, 0.6% magnesium, 0.2% sodium and 0.1% calcium. The pH of the diluted CCS was increased from 4.3 to 6.5 using 6 N NaOH.

Acclimation media were prepared by mixing Manna and CCS media in different ratios, along with glucose supplemented to the 30 g/L level. Then a comparison of the performances of the acclimated strains was done using hybrid media that were prepared by adding the dry components of Manna media (not including yeast extract) to the CCS medium.

Experimental design

To acclimate *S. paucimobilis* strain ATCC 31461 to grow and produce gellan on the CCS-based medium, it was grown on media prepared by mixing Manna and CCS media in different ratios, along with glucose supplemented to the 30 g/L level. Inoculum was prepared by growing a lyophilized culture in Manna medium for 72 h, then transferring 10 ml aliquots into 250 ml Erlenmeyer flasks containing 100 ml of medium. At first, *S. paucimobilis* was inoculated into media containing Manna: CCS ratios of 50:50, 25:75, and 10:90. These were incubated at 28°C and 250 rpm for at least 72 h, or until growth and thickening was evident. Cultures were transferred three times into the same Manna:CCS medium formulation, then progressively transferred to flasks with less Manna medium, until it was able to grow and produce gellan on the CCS medium alone, with 30 g/L glucose.

Growth and gellan production of the acclimated strain in Manna medium was then compared to that on the CCS medium and a hybrid media in which the dry components of Manna (except yeast extract) were added to the basal CCS medium. Reduced growth and/or gellan production in the hybrid Manna/CCS medium (compared to Manna medium) would indicate an inhibitory agent in CCS, while reduced performance in the CCS medium would indicate that CCS lacks a key component present in

Manna. Inoculation and incubation conditions were as above, and triplicate replications were performed.

After successful acclimation to the CCS medium supplemented with 30 g/L glucose, the optimal initial glucose concentration to maximize gellan production (and yet minimize residual glucose) was determined. The CCS medium was prepared as above, with glucose concentrations of 15, 30, 50, 100, 200, or 300 g/L. Inoculum consisted of 10 ml of a 72 h culture of *S. paucimobilis* grown on the CCS medium with the same glucose concentration. Shake flasks were incubated at 30°C and 250 rpm for 72-96 h, with triplicate replications. Samples were taken in 24 h increments to analyze residual glucose and to perform pour plates for viable cells. After incubation the remaining broth was processed to recover gellan.

A final series of trials were then conducted in a 5 L Bio-Flow III bioreactor using optimal conditions for gellan production (28°C, pH 6.5, 300-425 rpm, and 2.5 L/L/min aeration rate.) Here the CCS medium was compared to Manna medium (each supplemented with 30 g/L glucose) on the basis of cell growth and gellan production parameters. Triplicate replications were performed, each for a minimum of 85 h. Three liters of medium were inoculated with 5% of 36-48 h culture grown on either the Manna or CCS media. Samples were removed at 12 h intervals for plate counts on tryptic soy agar plates and HPLC analysis. For polysaccharide assays, 50 ml samples were removed from the bioreactor and frozen until analysis.

Gellan and dry cell weight determinations

A range of methods have been used to quantify gellan and dry cell weight. Fialho et al. [21] precipitated culture medium with 2.5 volumes

of cold ethanol (95%, v/v), washed the precipitate with ethanol, and then dried (24 h, 80°C) to determine gellan (combined with cell weight). To obtain cell-free gellan, Wang et al diluted culture broth in distilled water and then centrifuged to remove the cell pellet [38]. They added three volumes of ethanol (95% [vol/vol]) to the supernatant to precipitate gellan, which was recovered by centrifugation, and dried in a hot-air oven (60°C, 24 h). Unfortunately some gellan adheres to the cell pellet and is not recovered by this method. Therefore Manna et al. [9] boiled and pH adjusted the broth prior to diluting and centrifuging to remove cell mass. The cell pellet was then washed with dimethyl sulfoxide to remove adhering gellan, recentrifuged, and dried to obtain a gellan-free dry cell weight. Whole broth was then precipitated with 4 volumes of propanol to obtain total cell mass and gellan. Finally gellan was estimated by subtracting the dry cell weight.

A similar approach was used to that of Manna et al. [9], of precipitating both the cells and gellan, and then subtracting dry cell weight to determine gellan levels. However our approach to determining dry cell weight was somewhat different. In preliminary trials *S. paucimobilis* ATCC 31461 was grown up in a medium (Tryptic soy broth) that supported growth but not gellan production. Tryptic soy broth cultures were inoculated with a 5% inoculum, then incubated at 30°C for 48 h at 200 rpm. Triplicate samples were taken at 8 h intervals in the late exponential through early stationary phase (17-48 h). Viable cells were measured by plate counts in triplicate on solid tryptic soy agar using the pour plate method. Dry cell weight was determined by centrifuging and washing cell pellets, then drying to a constant weight at 80°C. A plot of dry cell weight/ml versus colony-

forming units/ml was prepared to determine the regression equation. Using this standard curve, the number of colony-forming units could be used to calculate the dry cell weight in subsequent trials. The calculated dry cell weight levels were subtracted from the dried polysaccharide-cell precipitate values to determine the actual gellan concentrations.

Gellan concentration was determined by autoclaving samples of broth at 121°C for 20 min. To the autoclaved broth, 2 volumes of cold 95% ethanol were added to precipitate the polysaccharide and cells, and the mixture was mixed vigorously. The precipitate was collected by centrifugation at 16,000 x g for 20 min at 4°C. The resultant pellet was dried to constant weight at 80°C, then the calculated dry cell weight was subtracted to determine gellan.

Analytical determinations

Samples to be analyzed via HPLC for sugars were filtered through sterile 0.2 µm nylon syringe filters into HPLC autosampler vials, which were frozen until analysis. In some cases, the high viscosity of the broth necessitated centrifuging at 16,000 x g for 20 min to obtain a supernatant that could be filtered. The analysis was conducted using a Spectraphysics HPLC system (San Francisco, CA) with an Aminex@HPX-87H column (Bio-Rad, Hercules, CA) and refractive index detector. The system was operated at 65°C, using a mobile phase of 4mM H₂SO₄ (degassed with helium) at a flow rate of 0.60 ml/min. Broth pH was measured with an Accumet 950 pH/ion meter from Fisher Scientific (Hampton, NH).

Results and Discussion

This study investigated whether condensed corn solubles (CCS), a low value ethanol

processing coproduct, could be used as an inexpensive production medium for the polysaccharide gellan by *S. paucimobilis*. Ingredient costs for Manna's medium would account for \$4.75-5.42 per Kg of gellan produced, compared to the current market price for gums such as guar (\$1.10- 1.87/Kg). In addition to medium cost, another important economic factor is the method used to recover gellan. For some applications, such as using gellan as a binding agent in soil erosion control products, cells or cell debris do not need to be separated from gellan. In unpublished work, we compared using gellan, gellan with cell biomass, and guar gum (control) for preparing soil protection barriers using paper pulp and fibers, and observed similar performance with all three binding agents. Therefore, in this study we also attempt to simplify the gellan recovery process to minimize processing costs, while producing a usable product.

Gellan recovery and correlation of dry cell weight and viable counts

In most gellan recovery methods, the broth is diluted with water prior to centrifugation so that a more clarified form of the polysaccharide remains in the supernatant. Dilutions of up to 1:12.5, including vigorous shaking and boiling, have been employed to disperse gellan from the cells [9]. However, it is likely that a portion of gellan is removed with the cell pellet, because even though it is extracellular, it can still bind to the cells. For example, Fiahlo et al. found that diluting with four or five parts water to facilitate removal of the cell pellet actually reduced gellan recovery [21]. Another unfortunate consequence of dilution is that the supernatant volume is increased, meaning that more solvent is needed to precipitate gellan. Some researchers have also evaluated use of solvents such as dimethyl sulfoxide [9] and 5%

MgSO₄ [33] to wash the cells to remove gellan. However this also increased the total volume of broth requiring centrifugation, and the volume of solvent for gellan precipitation. For example, up to 4 L of ethanol could be required to precipitate just 1 gram of gellan [9]. This obviously increases costs, and may prevent a process from becoming commercially feasible.

Our goal was simple and inexpensive recovery of gellan, thus, broth was not diluted to facilitate cell removal. Nor did we use solvents to enhance separation of cells and gellan. Instead we used a gellan recovery approach similar to that described by Manna et al. [9]. After autoclaving we precipitated acylated gellan in 2 volumes of cold ethanol and used centrifugation to recover the gellan and cell pellet. To quantify gellan, the cell population was determined, converted into dry cell mass, and then subtracted from the dry mass of gellan and cells. To ensure that only cell mass and gellan were recovered, we performed this assay on zero hour samples, both before and following inoculation. In un-inoculated samples we did not obtain any solids following ethanol precipitation and centrifugation. In samples taken immediately after centrifugation we obtained a small pellet of cell biomass only, since gellan production would not yet have occurred.

This required us to have previously developed a calibration curve of cell population vs dry cell mass in a medium (TSB) that supported *S. paucimobilis* growth but did not result in gellan production. Viable cell counts and dry cell mass were plotted to develop a linear calibration curve with a regression equation of $y^{-3E+07} e^{3933.6x}$ and a regression coefficient of $r^2=0.89$.

Acclimation to CCS-based medium

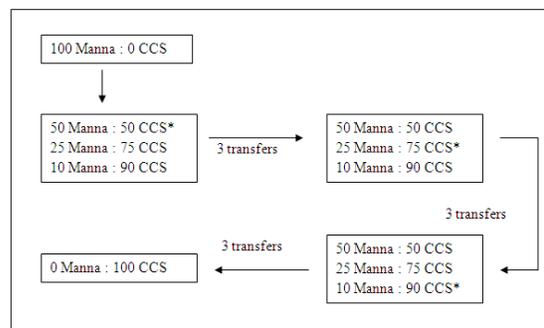


Figure 1. Acclimation of *S. paucimobilis* to Dry Mill CCS Based Medium^a.

^a Media formulations represent parts of defined Manna medium mixed with parts of CCS medium.

Acclimation of *S. paucimobilis* to the CCS medium followed the pattern shown in Figure 1. *S. paucimobilis* grown in the defined medium was used to inoculate flasks with progressively higher ratios of dry mill CCS medium. After 96 h, the flask which showed the greatest increase in viscosity (denoted by an asterisk) was transferred into the same concentration of hybrid medium for further acclimation. After three consecutive transfers in the same medium, the culture was again transferred into flasks containing even higher levels of CCS. This cycle was repeated until *S. paucimobilis* could grow and produce gellan on CCS medium exclusively. This stepwise progression took approximately six weeks, at which point the acclimated strain produced up to 5.8 g/L gellan. To further improve gellan production, *S. paucimobilis* was subcultured on the CCS medium, and reached ~11 g/L gellan after several more weeks. Culture pH remained consistently between 6.2-6.6 in these trials, so pH adjustment was not necessary.

Evaluation of Manna, CCS, and hybrid media

Growth and gellan production of the acclimated strain in Manna medium [9] was compared the CCS medium and a hybrid medium made by

Table 2. Parameters from Scleroglucan Production Trials using Manna Medium, CCS-based Medium, and hybrid CCS: Manna minerals medium.

| | Time (h) | Manna ^a | CCS ^b | CCS:Manna minerals ^c |
|------------------------------|----------|--------------------|------------------|---------------------------------|
| Viable Count (CFU/ml) | 96 | 6.7E9 ± 3.7E7 | 2.0E10 ± 2.4E7 | 2.3E10 ± 1.7E7 |
| Gellan (g/l) | 96 | 6.0 ± 0.9 | 11.0 ± 0.4 | 11.1 ± 0.5 |
| Glucose (g/l) | 96 | 30.5 ± 0.7 | 22.7 ± 0.8 | 21.3 ± 0.7 |

^a Manna is the defined medium for gellan production [9].

^b CCS consists of 58.6 g/L CCS and 30 g/L glucose.

^c CCS:Manna consists of the CCS medium with the addition of dry ingredients from Manna, except for yeast extract.

adding the dry ingredients of Manna to the CCS medium. Viable cell counts, gellan levels and glucose concentrations at 96 h are shown in Table 2. Viable cell counts and gellan concentrations in both the dry mill CCS based medium and the CCS medium supplemented with Manna components were similar, and exceeded that of the Manna defined medium. Thus instead of being inhibitory or lacking some required component, CCS satisfied all the nutritional needs of *S. paucimobilis*, provided additional glucose was supplied. Based on its proximate analysis via AOAC methods [27, 28, 29], it was theorized that CCS would provide sufficient nitrogen. West and Strohfus had previously shown this to be true for two co-products of corn wet mill processing (ie. corn steep liquor and corn steep solids) [20]. They found that these co-products supported greater gellan production by ATCC 31461 than did ammonium nitrate or peptone (whether glucose or corn syrup was used as the carbon source).

Optimum glucose concentration in CCS-based medium

Shake flask trials were performed using the CCS based medium to determine the optimal glucose concentration. The maximum cell population of 6×10^{10} CFU/ml occurred at 30 g/L initial glucose. Cell populations of $1-2 \times 10^{10}$ CFU/ml occurred when 0, 15, or 50 g/L glucose

were present, but were reduced by an order of magnitude at higher glucose levels, perhaps caused by osmotic repression. Figure 2 shows the maximum gellan and residual glucose levels. Even though the highest gellan concentration (16.9 g/L) was achieved at a starting glucose level of 200 g/L, the residual glucose level was far too high, resulting in a glucose utilization efficiency of only 70%. The next highest gellan titers (8.5-8.9 g/L) were at 30 and 200 g/L glucose, respectively, with both achieving glucose utilization efficiencies of ~38%. The lowest initial glucose levels resulted in the highest glucose utilization efficiencies (>95%), but only 4-5 g/L gellan. Gellan yield and productivity are shown in Figure 3. The medium containing 30 g/L glucose exhibited the highest gellan yield at 0.80 g/g, with an acceptable productivity level of approximately 0.09 g/L/h. Based on the combination of these parameters, 30 g/L initial glucose appears to be optimal.

Bioreactor comparison of CCS-based medium vs Manna's medium

Trials in a 5 L bioreactor were conducted to compare the CCS-based medium to the Manna medium (both at 30 g/L glucose) under optimal conditions of 28°C, pH 6.5, 300-425 rpm, and 2.5 L/L/min aeration rate. The results from a typical trial in the more refined Manna medium are shown in Figure 4. The maximum viable cell population of all three replicate trials averaged

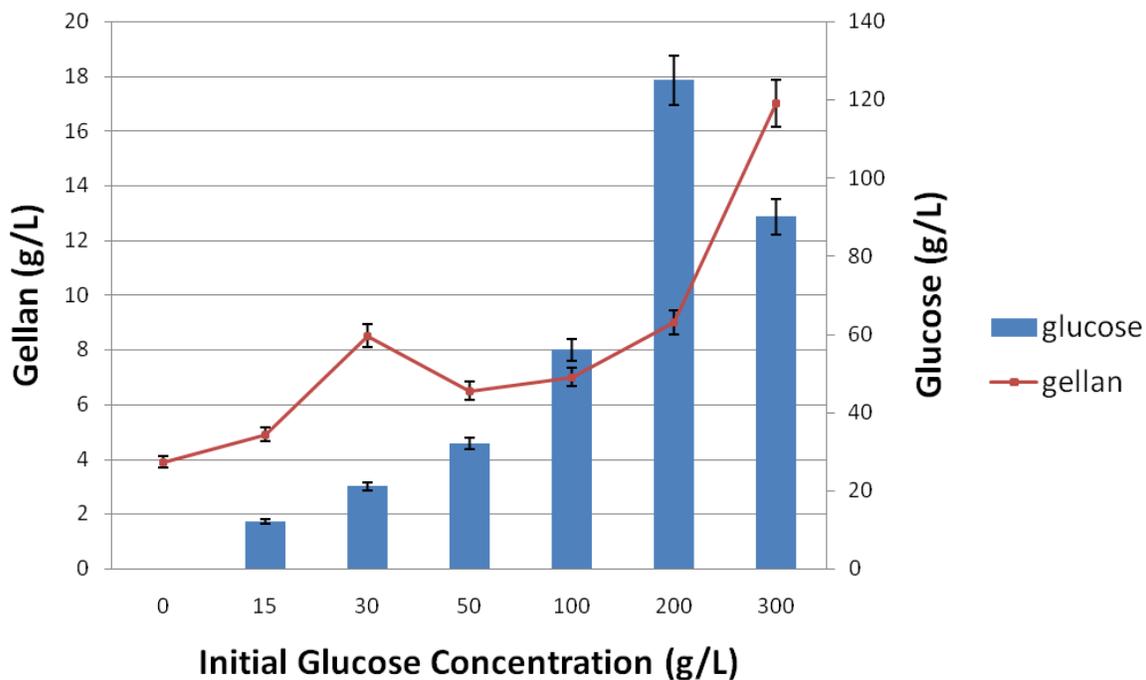


Figure 2. Effect of glucose concentration on gellan production and residual glucose levels using *S. paucimobilis* in CCS-based medium.^a

^aError bars represent one standard deviation.

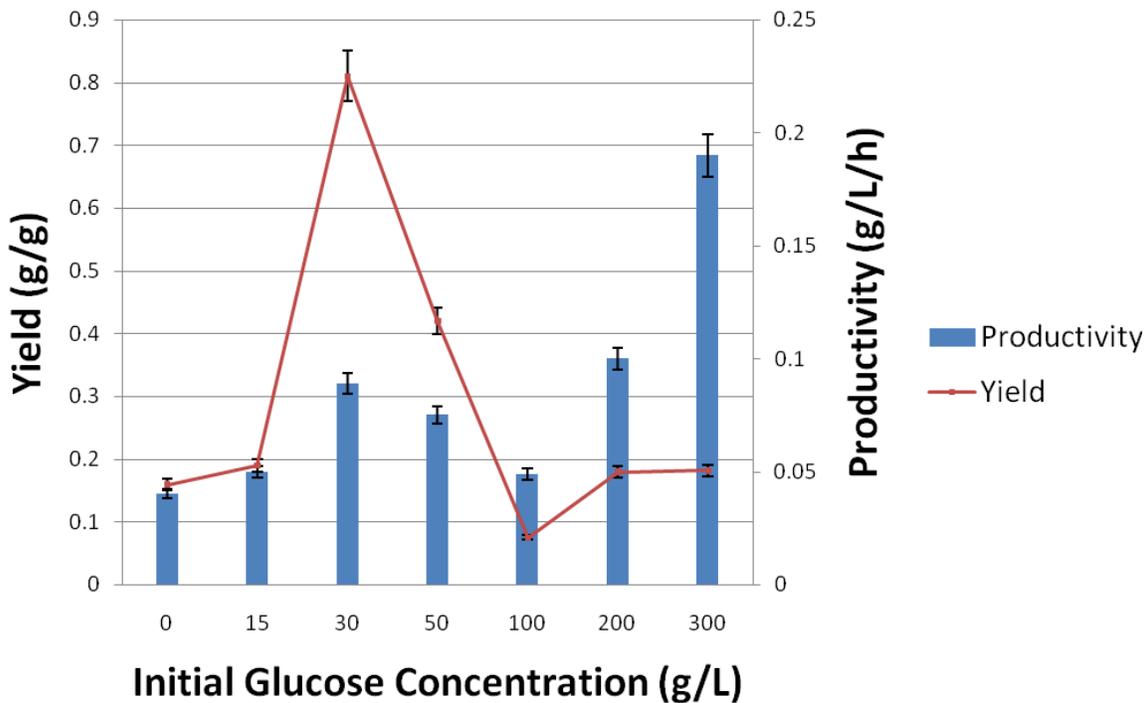


Figure 3. Effect of glucose concentration on gellan yield and productivity using *S. paucimobilis* in CCS-based medium.^a

^aError bars represent one standard deviation.

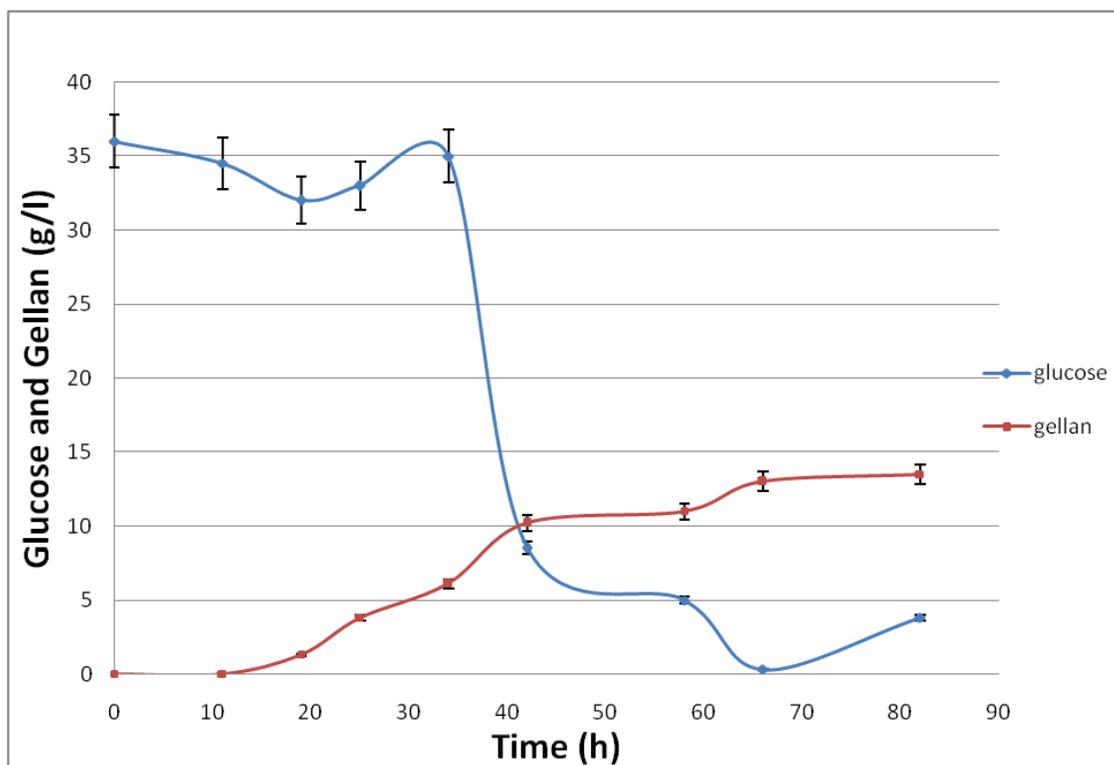


Figure 4. Typical Bioreactor Trial using of Defined (Manna) Medium^a.

^aError bars represent one standard deviation.

3.2×10^8 colony forming units/ml at 42 h while the glucose level dropped to approximately 3.2 g/L by the end of the experiment. Gellan levels reached an average of 10.1 g/L at 42 h and slowly rose thereafter to 13.6 g/L at 82 h. While higher viable cell numbers were reached in the shake flask trial (5.55×10^9 colony forming units/ml), gellan concentrations were higher in the bioreactor. This improvement was likely due to the higher rate of aeration and agitation that can be achieved in the 5 L bioreactor compared to conditions achieved in shake flasks.

Figure 5 shows the results from a trial with the CCS-based medium. Average viable cell counts in the three replicate trials using the solubles-based medium rose much more rapidly and achieved higher levels (4.4×10^{10} colony forming units/ml) than observed in the defined

medium. In contrast, glucose levels dropped more slowly and averaged 2.5 g/L at the end of the experiment. It is likely that *S. paucimobilis* preferentially utilized some of the other nutrients present in condensed corn solubles for growth and gellan production, thereby conserving glucose. The average gellan concentration of 13.4 g/L was higher than that observed in comparable trials in the defined medium.

For both media, use of the bioreactor resulted in increased gellan levels and viable cell counts. These results can be attributed to optimal aeration and agitation conditions being achieved. Data from bioreactor trials were used to calculate glucose utilization efficiency, productivity, and yields. Glucose utilization (90%+) and gellan productivities (0.18 g/L/h)

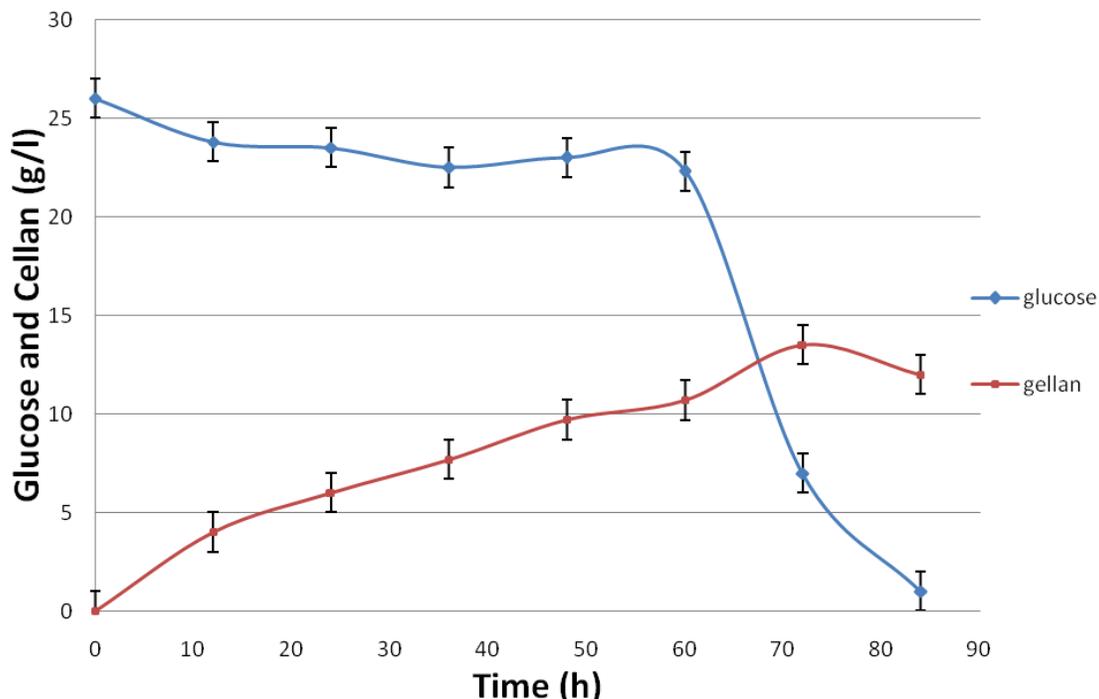


Figure 5. Typical Bioreactor Trial using of CCS-Based Medium^a

^aError bars represent one standard deviation.

Table 3. Production Costs for Gellan on Defined (Manna) Medium and CCS-based Medium.

| Component | Manna Medium | | CCS-Based Medium | |
|---------------------------------------|--------------|-------------------------------|------------------|-------------------------------|
| | Amount (g/L) | Cost (\$/1,000L) ^a | Amount (g/L) | Cost (\$/1,000L) ^a |
| Glucose | 30 | 23.64 | 30 | 23.64 |
| K ₂ HPO ₄ | 0.5 | 3.25 | -- | -- |
| MgSO ₄ | 0.1 | 5.45 | -- | -- |
| NH ₄ NO ₃ | 0.9 | 28.04 | -- | -- |
| Peptone | 0.5 | 15.00 | -- | -- |
| Salt solution ^b | 1.0 ml | 0.54 | -- | -- |
| CCS | -- | -- | 240 | 7.28 |
| Total Cost/1,000 L^c | | \$66.55 | | \$30.92 |
| Cost/Kg gellan^d | | \$4.75 | | \$2.58 |

^a Costs estimated based on bulk prices (Univar, USA, St. Paul, MN; Sigma Aldrich, St. Louis, MO). Hydrolyzed corn starch used to replace glucose, autolyzed yeast used to replace yeast extract.

^b Composition listed in materials and methods section.

^c Estimated costs of ingredients to prepare 1,000 L of fermentation broth.

^d Medium costs per Kg of gellan based on yield of 12 g dry gellan/L broth

were similar for both the Manna medium and the CCS-based medium, however the latter resulted in significantly higher gellan yields (0.57 g gellan/g glucose consumed). The gellan

productivity commonly cited in the literature is 0.45 g/g [34, 35]. The higher yields observed in the CCS-based medium may be due to *S. paucimobilis* utilizing other complex nutrients in

the solubles for growth, thereby conserving glucose for gellan production. For comparison, Manna medium resulted in a gellan yield of 0.45 g/g and productivity of 0.21 g/L/h.

Conclusion

The main objective of this study was to reduce the production costs of gellan from *S. paucimobilis* by replacing the expensive laboratory medium and using a more simplified recovery method. By acclimating the organism to grow on CCS based medium, the cost of the the medium for gellan production was reduced from \$4.75 per Kg gellan produced on defined (Manna) medium to \$2.58 per Kg (Table 3). These costs assume a gellan concentration of 12 g/L after 60-72 h of incubation. Improvements in gellan yields have the potential to further reduce production costs, making it more competitive with other exopolysaccharides. Gellan yields up to 14 g/L have been reported on the defined medium [36], whereas yields of up to 146 g/L have been reported for other more extensively studied exopolysaccharides like xanthan [9, 37]. Further research could address addition of specific amino acids and/or proteases, along with optimizing the carbon to nitrogen ratio of the medium.

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