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Biosurfactants from Potato Process Effluents

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Abstract

High-solids (HS) and low-solids (LS) potato process effluents were tested as substrates for surfactin production. Tests used effluents diluted 1:10, unamended and amended with trace minerals or corn steep liquor. Heat pretreatment was necessary for surfactin production from effluents due to indigenous bacteria, whose spores remained after autoclaving. Surfactin production from LS surpassed HS in all cases. Surfactin yields from LS were 66% lower than from a pure culture in an optimized potato starch medium. LS could potentially be used without sterilization for surfactin production for low-value applications such as environmental remediation or oil recovery.

Index Entries: *Bacillus subtilis*; biosurfactant; surfactin; alternate feedstock; potato; enhanced oil recovery.

Introduction

Low aqueous solubility and dissolution rates of hydrophobic organics limit both biodegradation and enhanced oil recovery *(1–6)*. Degradation rates of organic contaminants and oil recovery rates can both depend on mass transfer into the aqueous phase *(4–7)*. Surfactants increase the solubility and rate of transfer of organics into the aqueous phase by reducing interfacial tension. Chemical surfactants have been shown to enhance *in situ* recovery and removal of hydrocarbons, pesticides, and polychlorinated biphenyls *(4–6,8–10)*. Chemically synthesized surfactants, however, are often toxic *(11,12)* and are frequently expensive.

Biosurfactants have many properties that differentiate them from chemical surfactants, including biodegradability *(13)*, which is important in environmental and oil recovery applications *(14–16)*. Biosurfactants have a broader range of uses because optimum pH and salt concentration ranges are wider. Biosurfactants have been shown to assist in removal of pollutants

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Table 1
Substrate Characterization Data for Purified Potato Starch Control and Potato Process Waste Streams as Used in the Experiments[a]

	Starch source		
Carbon source (g/L)	Purified potato starch medium (PS)	1:10 HS effluent	1:10 LS effluent
Glucose	0.009	0.016	0.44
Soluble starch	4.88	4.69	14.6
Insolubles (starch + other)	0.001	16.2	6.57

[a]Concentrations are after autoclaving. HS and LS designations are based on the wt% of insolubles present.

from soil *(17)*, to lower interfacial tension *(18)*, and to enhance the recovery of natural resources *(14–16)*. Surfactin, a powerful antibiotic lipopeptide biosurfactant, has been studied extensively, and many reviews of this and other biosurfactants are available *(13,19–22)*. However, applications of biosurfactants are restricted to specialty markets because of high production costs. Cost-effective biosurfactants produced from low-cost renewable resources, such as agricultural processing residuals, could be used for resource recovery and environmental restoration. To do this, however, processing costs would need to be kept to an absolute minimum by eliminating expensive steps such as sterilization. Benefits would include reduction or elimination of waste streams and reduced use of toxic chemical surfactants. Previous research at the Idaho National Engineering and Environmental Laboratory (INEEL) has shown that production of surfactin, a lipoprotein biosurfactant from *Bacillus subtilis*, is technically feasible from purified potato starch *(14,23,24)*. This article evaluates the use of high-solids (HS) and low-solids (LS) potato processing waste streams for surfactin production by *B. subtilis*.

Materials and Methods

Potato Substrates

Potato process waste streams were obtained from a potato processing plant in southeast Idaho. Two streams were tested for surfactin production, including an HS and an LS stream. The HS waste was diluted 1:10 by weight with distilled water in the experiments, and the LS waste was diluted 1:10 by volume with distilled water. The waste streams were diluted to make a workable slurry for each. Control experiments were conducted using a purified potato starch (Sigma, St. Louis, MO), rendered soluble by boiling in distilled water for 30 min. Table 1 presents initial substrate data for the diluted potato process waste streams and the purified potato starch control (PS).

Cultures and Maintenance of Bacterial Strains

B. subtilis 21332 was obtained from the American Type Culture Collection (Manassas, VA). Several generations of cells were grown in maintenance broth *(25)*, and –80°C freezer stocks were prepared as previously described *(25)*. Seed inocula were prepared from the freezer stocks by adding one thawed tube of cells (2 mL) to 50 mL of Difco (Detroit, MI) nutrient broth and incubating for 18–24 h at 30°C and 150 rpm. The pH 6.0 nutrient broth *(26)* contained 5.0 g/L of pancreatic digest of gelatin and 3.0 g/L of beef extract. Typical seed cultures contained $4.0 \pm 0.6 \times 10^8$ cells/mL at the end of the incubation period.

Experimental Procedures

Controls

Controls for surfactin production included abiotic and biotic controls using purified starch as carbon source in an optimized medium. In each case, the pH 7.0 medium (PS) contained 5.0 g/L potato starch and trace minerals as follows: 0.396 g/L $(NH_4)_2HPO_4$, 0.015 g/L $FeSO_4 \cdot 7H_2O$, 0.00196 g/L $MnSO_4 \cdot H_2O$, 0.197 g/L $MgSO_4 \cdot 7H_2O$, 0.0010 g/L $CaCl_2 \cdot 2H_2O$, 5.678 g/L Na_2HPO_4, and 4.08 g/L KH_2PO_4. In each case, the medium was sterilized by autoclaving at 121°C for 20 min. Abiotic purified starch controls (A-PS) were not inoculated, whereas biotic purified starch controls (B-PS) were inoculated to 1 vol% with *B. subtilis* seed inoculum (see below for inoculation procedure).

After autoclaving of 1:10 diluted LS effluent (see below), it was observed that indigenous spore formers in the effluent survived the autoclaving. Since repeated autoclaving would degrade the starch and also be uneconomical in practice, we decided to include controls with LS medium diluted 1:10, pH 7.0, to test the effects of heat treatment on the competitiveness of inoculated *B. subtilis* with the indigenous microbes. The controls included an "abiotic" LS effluent control (A-LS) that was autoclaved but not inoculated with *B. subtilis*, and a "biotic" LS effluent control (B-LS) that was not autoclaved but was inoculated to 1 vol% with *B. subtilis* seed inoculum.

Effluents

All effluent-based media were initially adjusted to pH 7.0. The three media containing HS effluent were prepared with 10 wt% of process HS (wet cake as received from the potato processor) as the carbon source. Experiments were performed using inoculated unamended HS, inoculated HS containing trace minerals at the same levels as in PS medium (HS-M), and inoculated HS containing corn steep liquor (CSL) at 5 vol% (HS-CSL). The three media containing LS waste were prepared with 10 vol% of process LS (sludge as received from the potato processor) as the carbon source. Experiments with LS included inoculated unamended LS (LS), inoculated LS containing trace minerals at the same levels as in PS medium (LS-M),

and inoculated LS containing CSL added at 10 vol% (LS-CSL). Each effluent medium was autoclaved for 20 min at 121°C before use.

The batch tests were performed in triplicate in 250-mL Erlenmeyer flasks on a gyratory shaker at 30°C, 150 rpm, for 72 h. Each flask initially contained 125 mL of medium and was inoculated with 1.25 mL (1 vol%) of *B. subtilis* seed inoculum to begin the experiment. Homogeneous 8-mL samples were withdrawn at approx 0, 4, 8, 12, 24, 48, and 72 h for analysis. Five milliliters of each sample were frozen at –80°C in a tared tube for analyses of surface tension and insolubles. The absorbance at 550 nm was measured on the remaining 3 mL or the appropriate dilution, using the appropriate medium blank for each. Cells were then removed by centrifugation for 3 min at 5000*g*. The supernatant was transferred to a clean tube, sealed, and stored at 4°C for glucose and soluble starch analyses.

Analytical Methods

Cell Numbers

Cell numbers in serial dilutions of a stationary phase seed culture were determined using direct count techniques. The absorbances at 550 nm of these dilutions were then measured. A linear relationship between cell number and the absorbances at 550 nm was derived using linear regression and used to estimate cell numbers in actively growing culture samples. After the initial absorbance measurement, each actively growing culture sample dilution was then filtered through a 0.22-μm membrane, and its absorbance was measured again to provide the blank for its respective reading.

Substrate Concentrations

Glucose

Glucose was measured using a YSI Model 2700 Glucose Analyzer (Yellow Springs Instrument, Yellow Springs, OH). Samples were first centrifuged for 3 min at 5000*g* to remove cells and particulates.

Soluble Starch

Soluble starch was estimated using the phenol-sulfuric acid assay for total carbohydrates *(27)*. Soluble starch, assumed to be linear, was estimated from the total reducing carbohydrate concentration and the glucose concentration as follows:

$$\text{Soluble Starch (g/L)} = [\text{Total Carbohydrates (g/L)} - \text{Glucose (g/L)}] \times (162\ \text{g/mol})/(180\ \text{g/mol})$$

The ratio 162/180 accounts for the loss of one water molecule per glucose molecule as free glucose is polymerized to starch.

Insolubles

The 5-mL frozen samples were thawed, mixed, and centrifuged for 8 min at 5000*g*. The supernatant was used for surface tension measurement, and the pellet (containing starch particles, cells, and other solids) was refro-

zen at –80°C and lyophilized to dryness. The estimated weight of cells in each sample was then subtracted from the pellet weight, assuming an average per-cell mass *(28)* of 10^{-12} g/cell. The estimate for insolubles (starch plus other solids) was calculated by difference, recognizing that small amounts of water-soluble solids would also have carried over from water entrained in the insolubles.

Surface Tension

Surface tensions were measured by video image analysis of inverted pendant drops as previously described *(29)*. All measurements were made on cell- and solid-free supernatants obtained by centrifugation.

Surfactin Isolation and Critical Micelle Concentration

Surfactin was isolated by precipitation *(30)*, except that the crude powder was not washed with methylene chloride or filtered. The pH of the cell-free supernatant was adjusted to 2.0 by addition of concentrated HCl, precipitating the surfactin. The precipitate was collected by centrifugation, resuspended in nanopure water, and its pH adjusted to 7.0. The suspension was then frozen at –80°C and quantitatively lyophilized to dryness. The crude lyophilized powder was then used to estimate the Critical Micelle Concentration (CMC), in nanopure water, as previously described *(31,32)*.

Fourier Transform Infrared Spectroscopy

To confirm the presence of surfactin in the cultures, Fourier Transform Infrared Spectroscopy (FTIR) spectra of purified surfactin (Sigma; cat. no. S3523) and the crude precipitate from an LS culture were measured using a Nicolet (Madison, WI) Magna 750 FT-IR spectrophotometer equipped with an MCT-B (HgCdTe midband) detector. Transmission FTIR spectra of the pellets were averaged from 50 scans measured from 4000 to 400 cm^{-1} with 4-cm^{-1} resolution. A KBr background spectrum was subtracted from each spectrum. Measurements were performed on KBr wafers containing 2.5 wt% of pure surfactin or 3.8 wt% of crude precipitate in KBr, respectively.

Results

Cell Growth

No growth was seen in A-PS controls. Cell numbers in the B-PS control lagged behind those in B-HS and B-LS cultures regardless of additions. The B-PS control exhibited a 4- to 8-h lag, a characteristic not seen in either the HS or LS cultures. The B-LS control began with a much higher initial cell number than the other media, whereas the A-LS control started with a substantial initial cell count even though it had been autoclaved. All cultures were in late log phase at 12 h, and stationary phase was reached by 24 h. Table 2 presents specific growth rates for all media.

The LS media gave higher initial growth rates and a higher cell number at stationary phase than the other media. The addition of mineral salts to HS slightly increased cell growth, whereas addition to LS slightly

Table 2
Specific Growth Rates During Log Phase Growth[a]

Culture medium[b]	Autoclaved	Inoculated with *B. subtilis*	Medium addition	μ (h^{-1})
A-PS	Yes	No	Control	0.000
B-PS	Yes	Yes	Control	0.100
A-LS	Yes	No	None	0.085
B-LS	No	Yes	None	0.418
HS	Yes	Yes	None	0.272
HS-M	Yes	Yes	Minerals	0.341
HS-CSL	Yes	Yes	CSL	0.436
LS	Yes	Yes	None	0.447
LS-M	Yes	Yes	Minerals	0.406
LS-CSL	Yes	Yes	CSL	0.529

[a]Log phase ended in the 8- to 12-h range. Growth rates (μ [h^{-1}]) shown are for total bacteria in the cultures. The A-LS rate (autoclaved) is thus for indigenous bacteria from spores, and the unautoclaved B-LS rate is for inoculated *B. subtilis* and indigenous bacteria. PS media are pure cultures of *B. subtilis*, and all others are autoclaved, inoculated cultures.

[b]A-PS, Abiotic purified starch control; B-PS, biotic purified starch control; A-LS, "abiotic" LS effluent control; B-LS, "biotic" LS effluent control; HS, HS effluent medium; HS-M, HS medium with minerals added; HS-CSL, HS medium with CSL added; LS, LS effluent medium; LS-M, LS medium with minerals added; and LS-CSL, LS medium with CSL added.

decreased growth rates; the final cell number in each case was the same as that seen with unamended media, on the order of 10^9 cells/mL.

Substrates

Table 3 presents time courses of the carbohydrates in the B-PS, B-LS, LS, and HS cultures; included are glucose, soluble starch, and estimated insolubles. There were few qualitative differences in the time courses in the amended cultures; all differences correspond with slightly higher growth rates.

Glucose

B-PS controls contained essentially no free initial glucose. LS cultures contained about 0.5 g/L of initial glucose, except for the LS-CSL culture, which contained nearly 2 g/L of glucose from added CSL. HS cultures contained very little free glucose, with the exception of the HS-CSL culture. Glucose accumulation in all biotic cultures began near the onset of stationary phase, with the exception of CSL-amended cultures. In these cultures, glucose remained constant and decreased to near zero at 72 h.

Soluble Starch

B-PS controls initially contained all soluble starch. LS cultures contained about 14 g/L of initial soluble starch. The LS-CSL culture showed a calculated value of soluble starch of 22 g/L, indicating 9 g/L of reducing

Table 3
Time Courses of Starch-Related Substrates
for B-PS, B-LS, and HS and LS Effluent Media[a]

	Concentration (g/L)						
Culture medium	0 h	4 h	8 h	12 h	24 h	48 h	72 h
Glucose							
B-PS	0.009	0.003	0.005	0.002	0.005	0.379	0.452
B-LS	1.29	1.15	0.945	0.267	0.251	0.223	0.045
HS	0.0160	0.0010	0.0093	0.0090	0.161	0.398	0.260
LS	0.440	0.442	0.473	0.543	1.80	1.19	0.0330
Soluble starch							
B-PS	4.88	4.67	4.83	4.63	4.40	0	0
B-LS	18.9	18.4	21.6	14.7	6.14	10.4	6.25
HS	4.69	4.65	5.30	4.64	4.04	3.46	3.62
LS	14.6	14.4	14.5	15.4	9.64	0	0
Estimated insolubles							
B-PS	0.001	2.67	3.00	0.598	1.35	0.268	1.01
B-LS	12.2	6.59	6.00	6.51	6.34	3.92	4.52
HS	16.2	6.78	6.18	5.89	4.96	4.22	3.45
LS	6.57	4.10	3.63	3.56	4.42	3.27	4.12

[a]All media except B-LS were autoclaved before use. Differences in B-LS and LS media at time zero are attributed to this and to variations in process effluent composition from month to month. Refer to Table 2 for abbreviations.

sugars was added in the CSL (added to 10 vol%). HS cultures initially contained 4 to 5 g/L of soluble starch. The HS-CSL culture showed a calculated value of soluble starch of 7.5 g/L, indicating 4 g/L of reducing sugars was added in the CSL (5 vol%). Finally, the B-LS control contained about the same initial level of soluble starch as the other LS media.

Soluble starch consumption in the B-PS control indicates that glucose accumulation after the onset of stationary phase was due to starch degradation. Soluble starch stayed essentially constant as cell numbers increased, and then dropped quickly to zero. HS media showed similar trends in all cases, except that the soluble starch concentration did not drop completely to zero over the course of the 72 h. LS media exhibited similar trends. All CSL-amended media deviated from these trends, presumably because of the large amount of free glucose and nonglucose reducing sugars added in the CSL.

Insolubles

In all cases the correction applied to the lyophilized dry weight to subtract the cells was negligible. The B-PS control initially contained no insoluble starch, although a measurable amount of insoluble material (after subtraction of cell mass) remained in samples after freezing. HS media contained an estimated 16 to 17 g/L of insolubles, and addition of 5 vol% CSL

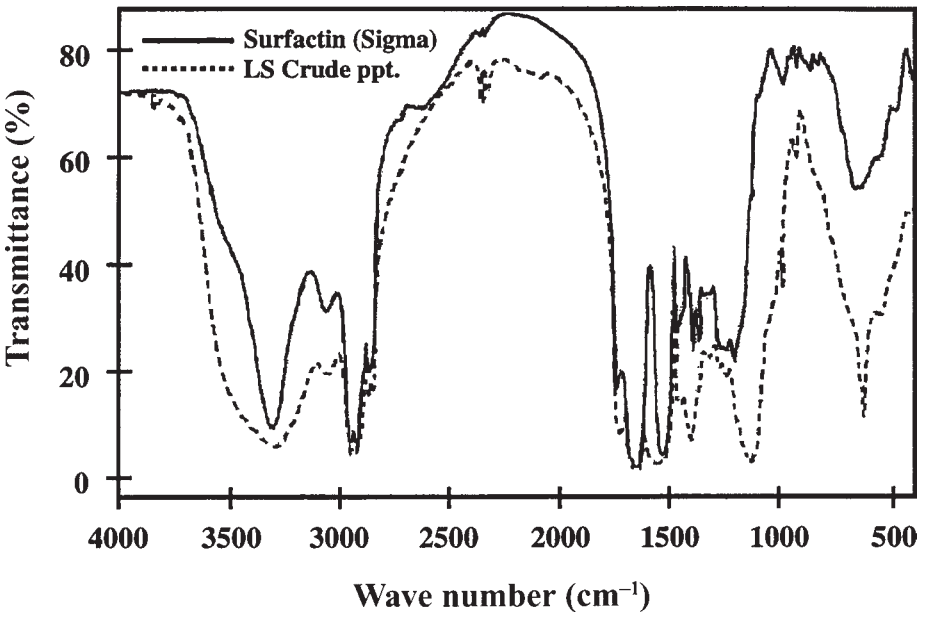

Fig. 1. Transmission FTIR spectra of purified commercial surfactin (Sigma) and the crude precipitate from the LS experiment. ppt., precipitate.

added another 18 to 19 g/L of insolubles. LS media initially contained about 7 g/L of insolubles, and addition of 10 vol% CSL increased the insolubles content of the medium to 35 g/L. This value is similar to that from the HS-CSL medium, indicating either that there was incomplete mixing of the CSL before addition to the diluted HS effluent or that the sampling of those flasks was not homogeneous.

The HS and HS-M cultures also showed some degradation of insoluble starch over the course of the reaction. The insolubles dropped to about 5 g/L about 8–12 h into the experiments. Similar results were observed with LS and LS-M media. The amount of insolubles left on entering stationary phase in the LS media essentially matched that seen in HS media, suggesting that the remaining solid may not be starch or that it may be starch that is difficult for the cells to degrade.

Fourier Transform Infrared Spectroscopy

Figure 1 shows the FTIR spectra of purified commercial surfactin and the LS crude precipitate. It is clear from these spectra that the biosurfactant produced was surfactin. The FTIR spectrum of the crude precipitate was not as sharp as that of the purified surfactin because of impurities.

Surface Tension

Table 4 presents the surface tensions of the cultures at 0, 24, and 72 h, along with crude surfactin yields and CMCs. Surfactant was produced only

Table 4
Comparison of Surface Tensions and Surfactin Yields[a]

Culture medium	Surface tension (mN/m)			72-h Yield data			
	$t = 0$ h	$t \approx 24$ h	$t \approx 72$ h	Crude precipitate concentration (ppm)	Crude precipitate Critical Micelle Concentration (ppm)	Surfactin yield (g/L of medium)	Surfactin yield (g/g of carbon)[b]
NP H_2O	71.3	71.3	71.3	0	ND	0	0
A-PS	69.3	69.3	69.3	0	ND	0	0
B-PS	66.0	57.7	29.1	2200	231	0.24	0.156
A-LS	58.5	65.2	56.0	ND	ND	ND	ND
B-LS	63.4	63.4	66.4	870	∞	0	0
HS	56.1	38.1	34.1	1900	500	0.097	0.0160
HS-M	48.3	32.2	29.0	2100	366	0.14	0.0103
HS-CSL	47.1	54.1	33.6	1900	353	0.13	0.0165
LS	51.2	28.5	25.6	3600	228	0.39	0.0504
LS-M	48.3	27.2	26.2	4100	ND[c]	0.44	0.0617
LS-CSL	45.5	52.0	49.0	1800	ND	0.19	0.0104

[a]Concentrations and Critical Micelle Concentrations are for crude precipitates, and surfactin yield is calculated from these data assuming a pure surfactin Critical Micelle Concentration of 0.025 g/L *(19)*. ND, not determined; NP, nanopure. Refer to Table 2 for other abbreviations.

[b]Yield is per gram of carbohydrate carbon consumed.

[c]The CMC from the LS medium experiments was used for all LS-based surfactin yield calculations.

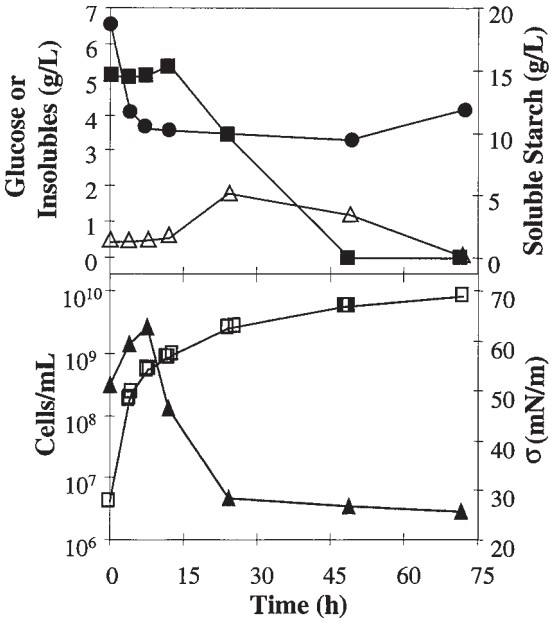

Fig. 2. Time courses of measured culture parameters for the LS culture. (■) Soluble starch; (●) insoluble starch; (Δ) glucose; (□) cells/mL; (▲) surface tension.

when *B. subtilis* was added to the cultures, and in the effluent media only when the media were autoclaved before use. The addition of trace minerals to the potato process effluents had little effect on surfactin production. The addition of CSL lowered surfactin production for both effluents. LS effluent was clearly a better substrate for surfactin production than HS effluent. The optimized B-PS control, however, produced an estimated threefold more surfactin per gram of carbohydrate carbon consumed than did the LS, at 0.0156 vs 0.050 g/g. These yields were estimated from the carbohydrate consumption data in Table 3, amounts recovered of crude precipitates, and the ratios of the pure surfactin Critical Micelle Concentrations (0.025 g/L) *(19)* and crude precipitate Critical Micelle Concentrations (Table 4). Note that changes in insolubles concentrations were presumed to be from starch hydrolysis for the purpose of these calculations. Adding PS medium levels of minerals to the LS to produce the LS-M medium increased this yield to only 0.062 g/g. Adding CSL decreased surfactin yield.

Figure 2 presents time courses of all parameters for the LS culture. It is clear that the initial production of surfactin in the cultures was growth associated, because the minimum surface tension occurred near the onset of stationary phase. Once the Critical Micelle Concentration was reached, further increases in surfactin concentration would not result in lowered surface tension. In Fig. 2, the Critical Micelle Concentration of the crude precipitate was reached at the point of attainment of minimum surface tension. The final concentration (3600 ppm) listed in Table 4 was measured at 72 h. Thus, it is not possible to attribute surfactin production in excess of

the Critical Micelle Concentration to growth or stationary phase, although production in late growth phase has been shown in the literature *(24,30,32)*.

Discussion

B. subtilis 21332 grew on all three potato substrates, regardless of additions of exogenous nutrients. Growth rates were higher in all HS- and LS-based media vs the B-PS control, and LS slightly outperformed the HS medium with respect to cell growth. Growth rates were marginally higher with added nutrients. However, the higher growth did not translate into lower surface tensions. In fact, the addition of CSL had a detrimental effect on surfactin production, whereas the addition of trace minerals had no effect on surface tensions. It is possible that the higher rates of growth seen in the LS media were due to higher initial amounts of free glucose present and to growth of contaminating indigenous bacteria. Indeed, LS media had an initial free glucose concentration of 0.44 g/L and outgrew the control. However, the HS media also outgrew the purified starch control but had only a little more free glucose in the medium than did the PS control. The addition of CSL did not affect the growth rates as much as expected.

Through the first 4–12 h of culture, the cells grown on each medium (except B-PS, which had a lag phase) were in log phase growth. It has been reported that the onset of surfactin production is in the mid-to-late log phase *(24,30,32)*, which we observed here. The growth rates on each medium would be expected to correspond to higher substrate utilization rates, and stationary phase would be expected to be reached near the time of substrate depletion. The final surfactant concentrations at 72 h were in all cases well in excess of their Critical Micelle Concentrations, whereas the minimum surface tensions were reached (which indicates that the Critical Micelle Concentrations were reached) near the onset of stationary phase. Thus, the bulk of the surfactant was produced in the late log phase or stationary phase. In all cases, most of the soluble starch degradation occurred during the stationary phase, whereas insoluble starch was degraded during the log phase. Glucose levels increased early in the stationary phase and declined after the soluble starch was depleted. This suggests that the free glucose content of the medium limited both early cell growth and later surfactin production. This behavior is illustrated for the LS culture in Fig. 2.

It is clear from the substrate utilization data that *B. subtilis* can express its α-amylase in these media. In all three media, *B. subtilis* was able to solubilize efficiently the insoluble starch and hydrolyze soluble starch to free glucose. The CSL-amended media deviated from this, perhaps because of the high initial concentration of free reducing sugars added in the CSL. Given this, it is likely that amylase production was inhibited by the carbohydrates added in the CSL. This was demonstrated in the experiments, since the CSL-amended media for both HS and LS exhibited higher initial growth rates than the other media (Table 2), but lacked the late glucose accumulation characteristic of amylolytic activity (data not shown).

These tests show that if the medium is first heat treated by autoclaving, the *B. subtilis* inoculum can compete, to some extent, for resources with germinating spores that survive, and produce surfactin. However, the spores that survive the heat treatment can germinate and grow substantially. This is clearly demonstrated by comparing the surfactin yields per gram of carbohydrate carbon consumed. When the *B. subtilis* cells were allowed to grow to high cell numbers on LS medium, surfactin was produced and the pH of the culture remained near 7.0 (data not shown). However, in the absence of added *B. subtilis*, when indigenous cells began to grow, the pH of the culture quickly dropped to 4.0 to 5.0 (data not shown), even 1 d after heat treatment. Without heating the medium to kill vegetative indigenous cells, the *B. subtilis* inoculum could not compete with the indigenous microbes. It is probable that the indigenous cells growing alongside *B. subtilis* consumed extra carbohydrate carbon and decreased the yields. This is shown by the B-LS culture, which was not autoclaved but was inoculated with *B. subtilis*.

Although it is suggested by the data, whether surfactin yields from LS effluent would increase to the level seen with PS medium if the indigenous cells and their spores were completely killed before inoculation with *B. subtilis* is not clear. It is improbable that cells growing at <25% of the rate seen in the LS culture (compare to the A-LS control in Table 2) could consume enough carbon to lower the surfactin yield by 66%. The addition of B-PS control levels of minerals to the LS effluent only slightly increased the yields from LS effluent, indicating a near-constant drain on the carbohydrate pool. This may indicate that the added minerals were consumed by the contaminating bacteria rather than by *B. subtilis*, or that there was simply no effect on the growth of either. This will be an important consideration because the value of the lost surfactin production could potentially be outstripped by the cost of complete sterilization of the medium, depending on the end use.

Conclusion

LS potato process effluents offer promise as carbon and nutrient sources for surfactin production by *B. subtilis*. The addition of CSL not only inhibited starch hydrolysis but also greatly lowered surfactin production. The addition of minerals to the medium had little effect on surfactin production from LS or HS potato process waste. The HS waste stream did not work as well for surfactin production and is not recommended for a surfactin production substrate. Heat treatment to kill vegetative indigenous cells was necessary to achieve surfactin production from both LS and HS effluents. However, the use of the effluents without complete sterilization, thereby allowing contaminating bacteria to remain, reduced the surfactin yield from the starch by 66% or more. Depending on the economics and the end use of the surfactin, complete sterilization may be unnecessary or economical.

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