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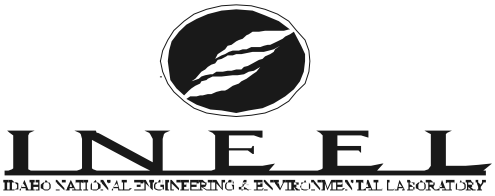

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SUMMARY

Pretreatment of low-solids (LS) potato process effluent was tested for potential to increase surfactin yield. Pretreatments included heat, removal of starch particulates, and acid hydrolysis. Elimination of contaminating vegetative cells was necessary for surfactin production. After autoclaving, 0.40 g/L of surfactin was produced from the effluent in 72 h, versus 0.24 g/L in the purified potato starch control. However, surfactin yields per carbon consumed were 76% lower from process effluent. Removal of starch particulates had little effect on the culture. Acid hydrolysis decreased growth and surfactant production, except 0.5 wt% acid, which increased the yield by 25% over untreated effluent.

KEYWORDS: *Bacillus subtilis*, biosurfactant, surfactin, alternate feedstock, enhanced oil recovery

INTRODUCTION

Numerous investigators have examined chemical surfactants to enhance in-situ removal of hydrocarbons, pesticides, and polychlorinated biphenyls *(1-3)*. Chemically synthesized surfactants, however, are not always environmentally benign *(4,5)* and are frequently expensive. Biosurfactants have been suggested as replacements for synthetic surfactants in environmental remediation, as well as for food emulsifiers, detergents, and for use in tertiary oil recovery *(6)*. Biosurfactants are potentially desirable versus synthetic surfactants on the basis of biodegradability, lowered toxicity, and the potential use of renewable substrates for their production *(6)*.

Surfactin is a powerful cyclic lipopeptide antibiotic biosurfactant produced by *Bacillus subtilis (7)*. Purified surfactin has an aqueous critical micelle concentration (CMC) of 25 mg/L and

lowers the surface tension to 27 mN/m *(7)*. Surfactin has been produced from glucose and other monosaccharides in amounts ranging from 0.1 to 0.7 g/L *(8-11)*. Foam fractionation techniques and iron or manganese addition can improve yields to 0.8 g/L *(7)*. However, high medium and separation costs limit its use in lower-value applications such as in situ bioremediation and enhanced oil recovery (EOR) *(7,12)*. However, high-purity surfactants are not needed for environmental or EOR applications, and thus substrate costs and product yields become overriding constraints *(12)*.

In a previous study, we showed that *Bacillus subtilis* ATCC 21332 produces surfactin from a low-solids potato processing effluent *(13)*. In this study, we examined the effects of several pretreatments on surfactin production from low-solids potato process effluent. Tested were the effects of autoclaving, removal of particulates, and dilute acid hydrolysis at several levels of severity. The data show that while it is necessary to initially kill contaminating cells in the effluent, no significant benefit in surfactin production is gained over the unamended effluent by further pretreatments. However, surfactin yield was increased slightly by dilute acid hydrolysis of the substrate before use.

METHODS

Potato Substrates. Low-solids potato process effluent was obtained from a southeast Idaho potato processor. Effluent slurry was diluted 1:10 by volume with nanopure water before use. Diluted effluent was pretreated and tested for surfactin production. Control experiments utilized both the diluted effluent and a purified potato starch obtained from Sigma.

Pretreatments. Pretreatments included autoclaving, filtration, and dilute acid hydrolysis. Autoclaving was done at 121 °C for 20 min. To prepare the filtered effluent, diluted LS was centrifuged for 10 min at 1180 $\times g$. The slurry in the lower half of the tube was discarded, and the supernatant slurry was filtered through P8 filter paper (Fisher, average pore size 20 μm). The filtrate (FLS) was used as the final substrate after autoclaving. Dilute acid hydrolysis of diluted LS was done by autoclaving after adding 1.42, 2.85, or 5.69 mL of concentrated H_2SO_4 to the undiluted

effluent and adding sufficient nanopure water to give 500 mL of 1:10 diluted LS containing 0.5, 1.0, or 2.0 wt% H_2SO_4. The acid hydrolyzed substrates were neutralized with 10 N NaOH before use. Acid hydrolyzed substrates were designated ALS½, ALS1, and ALS2, respectively. Untreated and pretreated media are summarized in Table 1 with their initial data.

Controls. Controls for surfactin production included abiotic and biotic controls using purified starch in an optimized medium. In each case, the pH 7.0 medium (PS) contained 5.0 g/L potato starch (Sigma), rendered soluble by boiling in distilled water for 30 min, and trace minerals as previously described *(13)*. All media were sterilized by autoclaving at 121 °C for 20 minutes. Abiotic controls (A-PS) were not inoculated, while biotic controls (B-PS) were inoculated to 1 vol% with *B. subtilis* seed inoculum.

Since indigenous spore-formers were found to survive autoclaving of diluted LS effluent *(13)*, controls employing diluted LS medium, pH 7.0, were included. The first of these controls was an "abiotic" control (A-LS) that was autoclaved but not inoculated with *B. subtilis*, called “abiotic” to indicate that it was not inoculated with *B. subtilis*. The second of these controls was a "biotic" control (B-LS) that was not autoclaved, but was inoculated to 1 vol% with *B. subtilis* seed inoculum, and was called “biotic” to indicate that *B. subtilis* was added to the culture. The “abiotic” control was used to show the growth of germinated bacteria in autoclaved samples and the lack of surfactin production in the absence of *B. subtilis*. The “biotic” control was used to determine the competitiveness of *B. subtilis* and the production of surfactin in the presence of high numbers of vegetative contaminating bacteria. Initial substrate data for diluted LS and its controls, and for the purified potato starch (PS) controls are also presented in Table 1.

Cultures and Maintenance.

Bacterial strains. *Bacillus subtilis* 21332 was obtained from the American Type Culture Collection (ATCC), and cultured as previously described *(13)*. Freezer stocks were prepared from cells grown in maintenance broth *(14)*. Seed cultures containing $4.0 \pm 0.6 \times 10^8$ cells/mL were prepared from the freezer stocks and were used to inoculate surfactin production tests. The seed

inocula were grown on Difco nutrient broth *(15)* as previously described *(13)*.

Experimental. The surfactin production tests were performed in 250 mL Erlenmeyer flasks on a gyratory shaker at 30 °C, 150 rpm, for 72 hours, as previously described *(13)*. Media used in the tests are listed in Table 1. All media were adjusted to pH 7.0 before autoclaving except for the acid pretreated substrates, which were adjusted just before use.

Analytical methods.

Cell numbers. Cell numbers were determined immediately after sampling using direct visual microscopic count techniques, as previously described *(13)*.

Glucose and pH. Glucose was measured, after removal of cells and particulates by centrifugation for 3 min at 5000 × *g*, using a YSI Model 2700 Glucose Analyzer (Yellow Springs Instrument, Yellow Springs, OH). Culture pH was measured using a standard pH probe.

Soluble starch. Soluble starch was estimated as previously described *(13)*, after removal of cells and particulates by centrifugation for 3 min at 5000 × *g*, and using the phenol-sulfuric acid assay for total carbohydrates *(16)*.

Insoluble starch. Frozen samples saved for surface tension analyses were thawed and centrifuged for 8 min at 5000 × *g*. Insolubles were estimated as previously described *(13)*, using the lyophilized pellet weight and the estimated weight of cells in each sample assuming an average per cell mass of 10^{-12} g *(17)*. The supernatant was used for surface tension measurement.

Surface tension. Surface tensions were measured by video image analysis of inverted pendant drops as previously described *(18)*. All measurements were made on cell-free supernatants obtained by centrifugation.

Surfactin isolation and Critical Micelle Concentration. Crude surfactin was isolated by precipitation *(9)*, as previously described *(13)*. The crude lyophilized powder was then used to estimate the Critical Micelle Concentration (CMC), in nanopure water, as previously described *(19,20)*.

RESULTS

Pretreatments. The effects of the pretreatments on initial substrate composition are summarized in Table 2. The B-LS control (unautoclaved) represents the LS medium without pretreatment. There were some small variations in the initial glucose and starch levels of undiluted LS effluent as obtained from the processor. Initial glucose in the B-LS medium was 1.41 g/L, with soluble starch and insolubles at 14 and 5.5 g/L, respectively. Autoclaving the effluent lowered the initial glucose by about 20%, increased the soluble starch by 18%, and nearly doubled the insolubles content. As expected, filtering the diluted LS before autoclaving had little effect on glucose content. However, the soluble starch content increased by 45%, and the total insolubles content was essentially unchanged. Acid hydrolysis increased the glucose content from hydrolysis of the starch, thereby decreasing the soluble starch content. Again, the insolubles content increased relative to the unautoclaved effluent.

Cell Growth. In all cases except the B-PS control, log phase growth was over by 12 h. For the B-PS control, 24 h were required. The specific growth rates for the log phase are presented along with the initial substrate data in Table 1. For LS-based media, growth cannot be specifically attributed to *B. subtilis*, since there were contaminating bacteria present (germination and growth of these bacterial spores was observable in the A-LS control). Thus, growth rates in Table 1 represent the sum of contaminant bacteria and *B. subtilis*.

Removal of particulates by filtration had little effect on growth. The specific growth rate averaged over 12 h for the FLS culture was slightly higher than that for the LS culture. Addition of acid to the LS medium before autoclaving increased the specific growth rate when 0.5 wt% H_2SO_4 was added. However, doubling the acid concentration decreased the specific growth rate relative to 0.5 wt%, although this was still a slight increase over the 0% acid medium (LS). Addition of acid to 2 wt% also had a detrimental effect on cell growth, decreasing the specific growth rate to below that for the LS medium.

Culture pH. In all autoclaved media inoculated with *B. subtilis*, culture pH remained essentially

constant around 7.0 over the first 48 h of the experiment (data not shown). After 48 h, all but the B-PS control culture showed an increase in pH to near 8.0; the B-PS control culture pH remained constant at 7.0 for the entire experiment. With autoclaving but without inoculation of *B. subtilis* (A-LS), the pH stayed constant at about 7.0 for 8 h and then decreased to 6.5. Finally, the inoculated, unautoclaved culture pH decreased to 4.5-5.0 over the first 8 h of culture, and remained low over the rest of the experiment.

Glucose Consumption. Glucose consumption in the controls and pretreated media is presented in Figure 1. The B-PS control culture initially used all glucose released from the added starch, but began to accumulate glucose to a small degree after 8 h. The A-LS culture showed an 8 h lag before use of the 1.3 g/L of free glucose, but eventually began utilizing much of the free glucose. Glucose in the B-LS culture quickly dropped to about 0.25 g/L over 8 h, and then remained relatively constant. The LS and FLS media essentially mirrored one another, first accumulating glucose over 12 h and then slowly utilizing the glucose over the remainder of the culture. Finally, the acid pretreated LS media showed 8-12 h lag times before utilization of the free glucose, but after the lag, each culture utilized the glucose at an essentially linear rate until it was gone.

Soluble Starch. Soluble starch consumption for all media is presented in Figure 2. In the B-PS control medium, there was a short lag of 4-8 h in degradation of soluble starch, with apparently linear degradation thereafter. The A-LS medium showed a slight increase in soluble starch over the first 4 h of culture, and again showed linear degradation afterward. The B-LS control displayed an initial increase in soluble starch, and then nonlinear degradation. The LS and FLS medium behaved similarly, peaking at 8 h. However, acid-pretreated LS media showed essentially a linear degradation of the starch over the entire culture.

Insolubles. The time courses of insolubles concentration in the cultures are presented in Figure 3. The B-PS control did not contain initial insolubles and so is not included. In all LS-based media, a fraction of the insolubles was quickly solubilized, leveling off after about 8 h. In all but the A-LS and FLS cultures, the final insolubles concentration was 5-6 g/L. The final A-LS insolubles

concentration was less than 1 g/L, and that in the FLS culture was 2-3 g/L. Most of the insolubles decrease took place while soluble starch was increasing, indicating solubilization of the starch fraction of the insolubles.

Surface Tension. The surface tensions of the effluent-based control media were initially in the range 60-65 mN/m. The surface tensions of the LS and acid hydrolyzed effluent media were in the range 50-55 mN/m. The B-PS control medium began with a surface tension of 72 mN/m. The B-PS control culture reached 31 mN/m in 24 h, but did not change much thereafter. The surface tension in the A-LS culture remained essentially constant, with a small drop after 24 h to about 55 mN/m. In the B-LS control, the surface tension increased slightly from 60 to 65 mN/m. The LS and FLS cultures again behaved identically, reaching 29 mN/m in 24 h and 25-27 mN/m after 72 h of culture. The behavior of the acid-pretreated media was slightly different that that of the LS culture, slowly increasing from the initial surface tension of 55 mN/m to 62 mN/m, and then decreasing to 29 mN/m at 48 h; no change in surface tension was seen after 48 h of culture.

Surfactin isolation and Critical Micelle Concentration. Surfactin recovery data and CMCs are shown in Table 3. The B-PS control produced 2200 mg/L of crude surfactant, and the B-LS control produced 870 mg/L. Since the surface tension did not change in the B-LS control, the 870 mg/L is an acid-insoluble fraction present in the LS effluent. This cannot be verified with the A-LS medium since no recovery was done. The FLS medium produced 4000 mg/L of crude surfactant versus 3600 mg/L from the LS medium. Somewhat less solid was recovered from the acid-pretreated media, at about 2700 mg/L for each.

The CMCs indicate that there was substantial carryover of non-surfactant acid-precipitable solids into the crude surfactant. The lowest measured CMC was 141 mg/L for ALS½, which indicates that about 17 wt% of the crude surfactant was surfactin. The ALS½ was the best pretreatment, producing nearly 0.50 g/L of surfactin at 72 h. The nearest result was for autoclaved LS, at about 0.40 g/L of surfactin. The B-PS control produced 0.24 g surfactin/L by 72 h. The B-PS control produced 0.154 g surfactin per g carbon consumed, compared to 0.037g surfactin for LS and

0.051 g from ALS½. Removing the large particulates from the LS decreased the yield from the effluent to 0.025 g surfactin/g carbon, and stronger acid pretreatments also substantially decreased surfactin yields from glucose.

DISCUSSION

PS Medium: Purified starch control. Growth of *B. subtilis* on PS medium was poorer than cell growth on LS effluents. The lag observed in cultures with low initial glucose occurs while the amylase system is induced, as verified by glucose and soluble starch data. The surface tension in the B-PS control culture reached 31 mN/m in 24 h, but did not change much thereafter. Crude surfactant recovery from the B-PS control was 2200 mg/L, with a CMC of 231 mg/L. The concentration of surfactin at 72 h, estimated as previously *(13)* from the CMCs of pure surfactin and of the crude precipitate, was 0.238 g/L, which is in the range 0.1-0.7 g/L previously reported from monosaccharides *(8-11)*. This suggests that much of the glucose consumed went to production of the amylase system. The yield per gram of carbon consumed was the highest from any of the media tested in this study, at 154 mg surfactin.

B-LS, A-LS, & LS Media: Effect of autoclaving. The A-LS and B-LS controls both supported growth, indicating significant contaminating microbial activity in the effluent. However, the purpose of autoclaving was not to sterilize the effluent but to minimize cost by simply allowing *B. subtilis* to successfully compete in the culture. After autoclaving, inoculated *B. subtilis* grew well and produced surfactant and so could apparently compete for resources. The contaminant bacteria were likely fermentative, since pH in the A-LS and B-LS controls quickly dropped to 4.5, well below that required by *B. subtilis* 21332.

Glucose in the B-LS control dropped over 8 h and remained constant thereafter, which correlates with the bottoming out of culture pH. Soluble starch was degraded in all cultures, so contaminant cells expressed amylase activity. Soluble starch degradation was slower in the A-LS culture than in the others, but was to a greater extent than in either the B-LS or the LS cultures. Both

B-LS and LS cultures initially showed increasing soluble starch, corresponding with high rates of degradation of insolubles. As in the other media, insolubles leveled off at 5-6 g/L, indicating a recalcitrant and/or non-starch fraction.

Surface tensions in the A-LS control did not change appreciably, verifying that surface tension changes in *B. subtilis*-inoculated cultures were attributable to *B. subtilis*. The surface tension also did not change in the B-LS control nor was there a measurable CMC. Since the pH of this culture quickly dropped to 4.5, the *B. subtilis* was not able to produce surfactin and may not have been able to grow significantly.

The LS medium produced 0.395 g/L of surfactin at 72 h, which was within the reported range for monosaccharides *(8-11)*, and above that observed for the B-PS control. However, the estimated yield of surfactin from carbon consumed was only 24% of that from B-PS medium, at 0.037 g surfactin. It is likely that medium additions or complete sterilization of the effluent could make up this yield loss. However, since the aim is to keep costs low, it is unclear whether the economics of the process through the final separation step would favor feedstock additions or treatments.

FLS Medium: Effect of filtration. The principle reasons for the filtration pretreatment were to remove large particulates that may serve as carriers for spores that survive autoclaving, and that could plug oil reservoirs if carried over to the surfactin product. Filtration had little effect on cell growth, as expected. The great majority of the particulates were clearly able to pass through the 20 μm filter paper, evidenced by the essentially unchanged insolubles concentration.

Culture pH, glucose consumption, and soluble starch consumption in the FLS medium all paralleled the LS medium. Insolubles consumption in the FLS medium was somewhat higher than that in the LS medium, ending at 2-3 g/L of insolubles. Thus, it is likely that some of the recalcitrant insolubles were removed during the filtration. The lack of differences in LS and FLS cultures suggests that removal of particulates from the diluted LS effluent before autoclaving had no effect. This was again seen in the surface tensions, which mirrored one another over the course of the runs.

However, the CMC of the crude surfactant from the FLS culture was substantially higher than that from the LS medium. The estimated surfactin concentration at 72 h was 25% lower than that observed from the unfiltered LS medium, and the yield of surfactin per gram of carbon consumed was 32% lower than from LS and 84% lower than from B-PS. Thus, it is preferable to leave the particles in the LS medium during surfactin production.

ALS Media: Effect of dilute acid pretreatments. Addition of a small amount of acid to the medium before autoclaving (0.5 wt%) increased cell growth rates, although higher acid concentrations adversely affected cell growth (Table 1). It is likely that the higher acid concentrations caused some decomposition of the glucose released, forming 5-hydroxymethyl-2-furfuraldehyde (HMF), levulinic acid, and formic acid *(21)*. These decomposition reactions are common in acid hydrolysis of cellulosic biomass *(22)*, and acid hydrolysis products of lignocellulose have been shown to be somewhat toxic to yeasts used for ethanol fermentations *(23)*. It is probable that similar decomposition products are formed during starch hydrolysis and that these products could be toxic to *B. subtilis* 21332.

Culture pH in the acid-pretreated LS media again mirrored that in the other LS-based media. The acid-pretreated LS media all showed 8-12 h lag times in glucose consumption, but after the lag each culture utilized the glucose at linear rate. Soluble starch consumption was also linear, indicating balanced glucose consumption and soluble starch degradation. There was no difference in the rates of soluble starch degradation with increasing severity of pretreatment, indicating that initial glucose concentrations from 2.3-5.3 g/L had little effect on amylase induction and production. Insolubles consumption in the acid-pretreated cultures again bottomed out near 5-6 g/L.

The CMC of the crude surfactant increased with pretreatment severity, indicating that more acid precipitable compounds were present after hydrolysis with higher amounts of acid. The 0.5 wt% acid treatment had the highest estimated surfactin concentration of any of the media tested, at 0.479 g/L, which is 68% of the highest reported value without foam removal (60% of that with foam removal). The 0.5 wt% acid treatment also had the highest surfactin yield from glucose, at 0.051 g

surfactin per gram of carbon consumed, as compared to 0.030 and 0.017 g/g for the 1 and 2 wt% pretreatments, respectively. This again suggests that an inhibitory product of the acid hydrolysis limits surfactin production, and also suggests that the lowered surfactin production seen in a previous work in which corn steep liquor (CSL) was added to the process effluent *(13)* was due to an inhibitory compound present in the CSL.

CONCLUSIONS

Autoclaving of the process effluent before use as a substrate for surfactant production is absolutely required if surfactant production is desired. If removal of particulates is necessary, this step would be better placed after surfactant production. Dilute acid hydrolysis of the diluted LS effluent with 1 wt% acid or higher, has a detrimental effect on growth, rate of production and total amount of surfactant produced. Pretreatment with 0.5 wt% acid modestly increased surfactin yield over untreated LS. All media performed poorly on a yield-per-carbon consumed basis when compared with the optimized control culture. While it is likely that medium additions or complete sterilization of the effluent could make up this yield loss, it is unclear whether the economics of the process through the final separation step would favor feedstock additions or complete sterilization. Further studies that include separation of the surfactin will be necessary to answer this question.

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TABLES

Table 1: Substrate characterization data for the purified potato starch control and for the pretreated potato effluents. The B-LS control, which was not autoclaved, is the untreated 1/10-diluted process effluent. Some variation between batches was seen in this stream. The final column is the observed specific bacterial growth rates at 12 h seen in experiments using each substrate. The cultures were still in log phase at 12 h except for the B-PS control. The growth rates are for total bacteria in the cultures.

Process Effluent[a]	Pretreatment	Inoculated With *B. subtilis*?	Glucose (g/L)	Soluble Starch (g/L)	Insolubles (g/L)	Specific Growth Rate (h^{-1})
Uninoculated Controls						
A-PS	Autoclaved	No	0.009	4.88	0.00	0
A-LS	Autoclaved	No	1.29	18.9	12.2	0.085
Inoculated Controls						
B-PS	Autoclaved	Yes	0.009	4.88	0.00	0.161
B-LS	None	Yes	1.41	14.0	5.50	0.418
Pretreated Substrates						
LS	Autoclaved	Yes	1.13	16.5	10.8	0.345
FLS	Filtered & Autoclaved	Yes	1.15	20.3	11.2	0.366
ALS½	0.5 wt% H_2SO_4 & Autoclaved	Yes	2.37	14.3	10.2	0.399
ALS1	1.0 wt% H_2SO_4 & Autoclaved	Yes	2.52	12.6	10.2	0.367
ALS2	2.0 wt% H_2SO_4 & Autoclaved	Yes	5.37	11.3	10.3	0.332

a Designations are: A-PS = Abiotic purified starch control; B-PS = Biotic purified starch control; A-LS = "Abiotic" low-solids effluent control; B-LS = "Biotic" low-solids effluent control; LS = Low-solids effluent medium; FLS = Filtered low-solids effluent medium; ALS½ = ½ wt% Acid-hydrolyzed low-solids effluent medium; ALS1 = 1 wt% Acid-hydrolyzed low-solids effluent medium; and ALS2 = 2 wt% Acid-hydrolyzed low-solids effluent medium. Unless otherwise indicated, all media were autoclaved.

Table 2: The effect of pretreatments on relative substrate concentrations in pretreated diluted LS effluent. All pretreatments included autoclaving the substrate at 121 °C for 20 minutes. The B-LS substrate was not autoclaved.

		Fraction of Initial (After Pretreatment)		
Pretreatment	Medium	Glucose	Soluble Starch	Insolubles
None	B-LS	1.00	1.00	1.00
Heat	LS	0.801	1.18	1.96
Filtered + Heat	FLS	0.816	1.45	2.04
0.5 wt% H_2SO_4 + Heat	ALS½	1.68	1.02	1.86
1.0 wt% H_2SO_4 + Heat	ALS1	1.79	0.900	1.86
2.0 wt% H_2SO_4 + Heat	ALS2	3.81	0.807	1.87

Table 3: Crude surfactant recovery, CMC, and yield for each pretreatment. Crude surfactant was recovered from the combined 72 h culture fluid from the three replicates.

	Recovered Solid (g)	Solid Concentration At 72 h (mg/L)	Measured CMC (mg/L)	Surfactin Yield At 72 h (g/L)	Surfactin Yield At 72 h (g/g carbon)
Nanopure H_2O	0	0	∞	---	0
A-PS	0	0	ND	0	0
B-PS	0.46	2200	231	0.238	0.154
A-LS	ND*	ND	ND	---	---
B-LS	0.21	870	∞	---	0
LS	0.76	3600	228	0.395	0.037
FLS	0.85	4000	340	0.294	0.025
ALS½	0.58	2700	141	0.479	0.051
ALS1	0.56	2600	265	0.245	0.030
ALS2	0.57	2700	458	0.147	0.017

* ND = Not determined

FIGURE LEGENDS

Figure 1: Glucose consumption versus time for growth on control and pretreated potato effluent. **(a)** Controls, autoclaving, and filtration pretreatments; symbols are (■) B-PS control, (▲) A-LS, (●) B-LS, (◊) FLS, and (×) LS. **(b)** Potato starch control and dilute acid pretreatments; symbols are (■) B-PS control, (×) LS (0% acid), (▲) ALS½, (●) ALS1, and (◊) ALS2.

Figure 2: Soluble starch versus time for growth on control and pretreated potato effluent. **(a)** Controls, autoclaving, and filtration pretreatments; symbols are (■) B-PS control, (▲) A-LS, (●) B-LS, (◊) FLS, and (×) LS. **(b)** Potato starch control and dilute acid pretreatments; symbols are (■) B-PS control, (×) LS (0% acid), (▲) ALS½, (●) ALS1, and (◊) ALS2.

Figure 3: Insolubles versus time for growth on pretreated potato effluent. **(a)** Controls, autoclaving, and filtration pretreatments; symbols are (▲) A-LS, (●) B-LS, (◊) FLS, and (×) LS. **(b)** Potato starch control and dilute acid pretreatments; symbols are (×) LS (0% acid), (▲) ALS½, (●) ALS1, and (◊) ALS2.

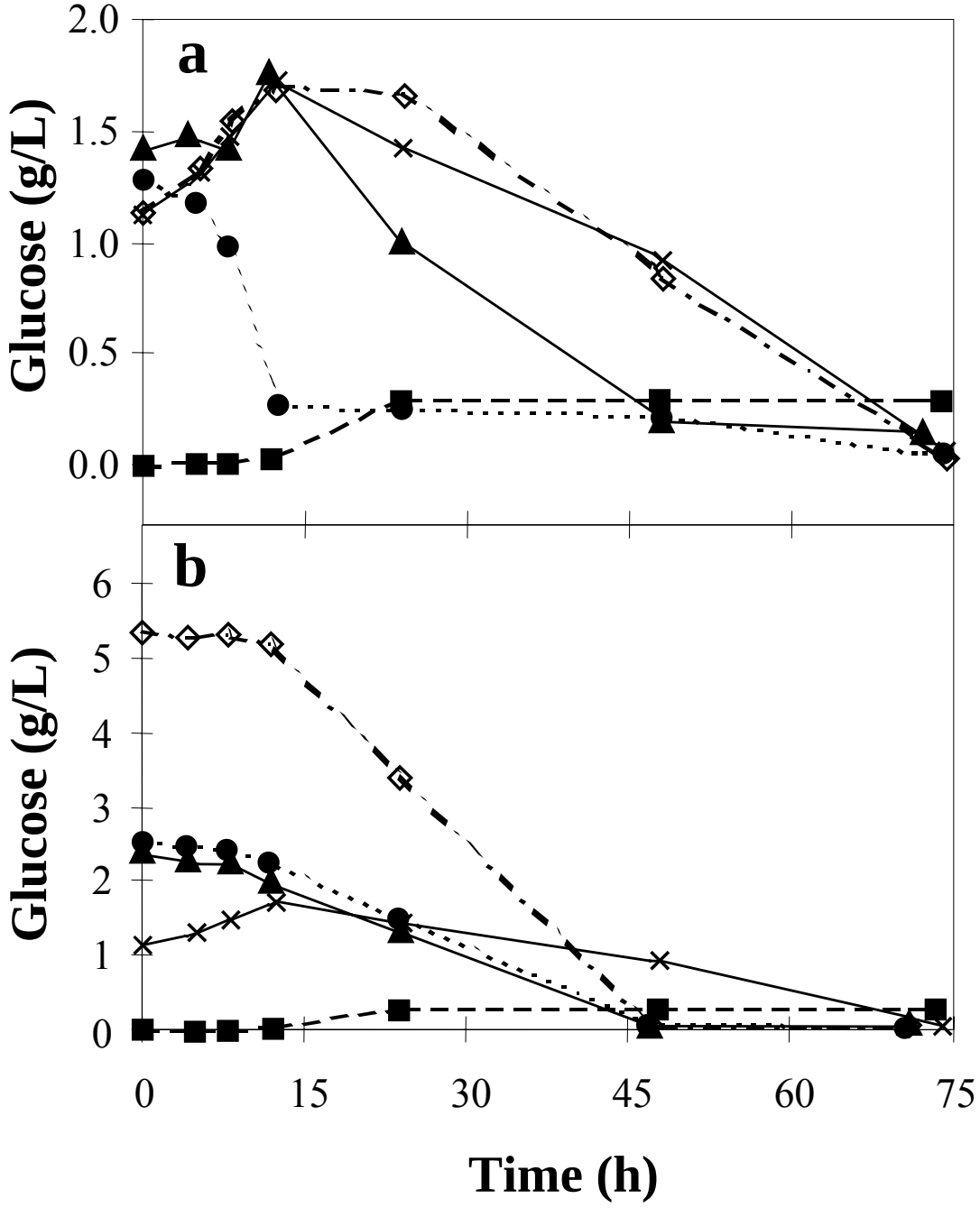

FIGURE 1 – THOMPSON, FOX, AND BALA

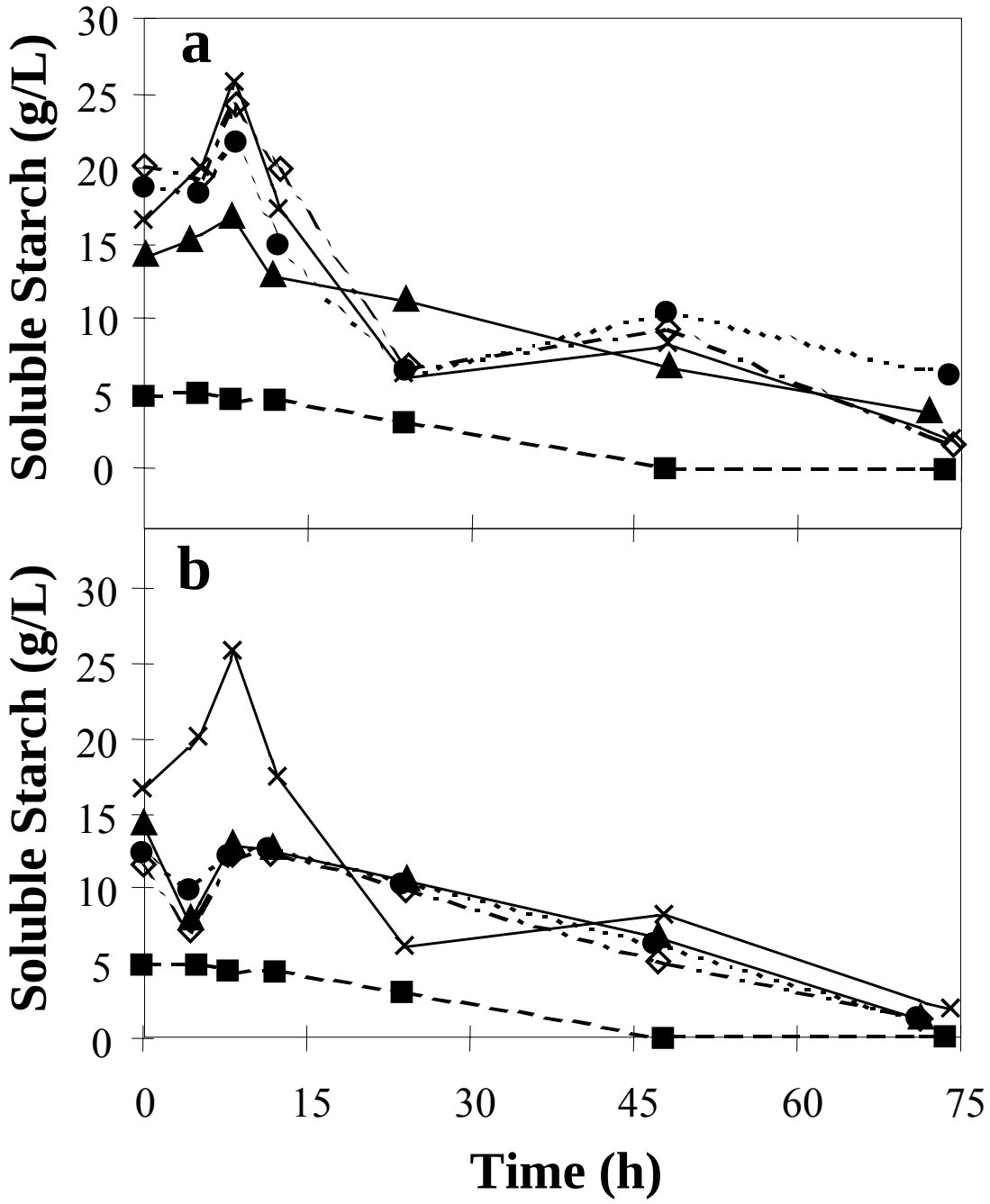

FIGURE 2 – THOMPSON, FOX, AND BALA

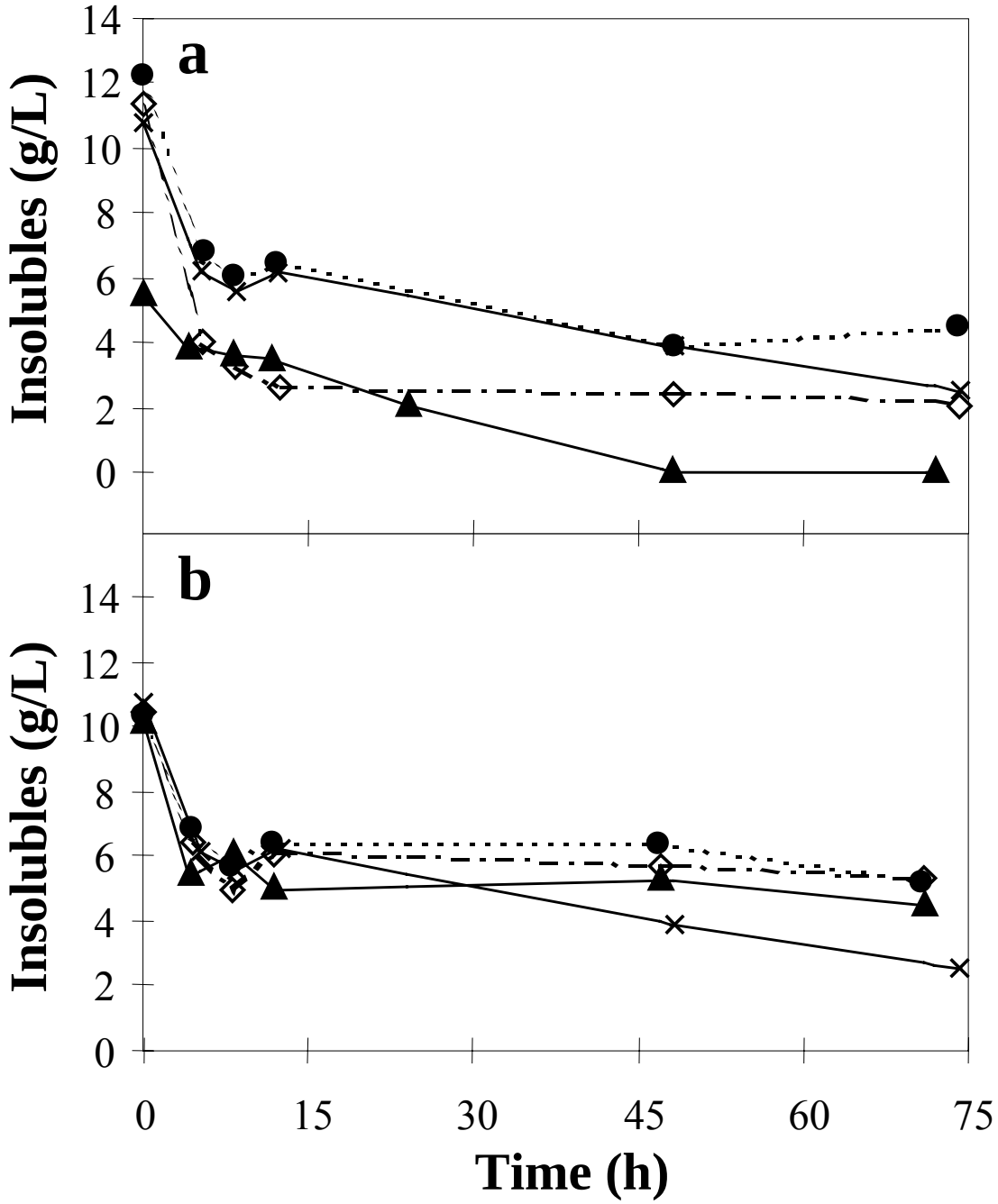

FIGURE 3 – THOMPSON, FOX, AND BALA