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Efficient Strategy to Alleviate the Inhibitory Effect of Lignin-Derived Compounds for Enhanced Butanol Production

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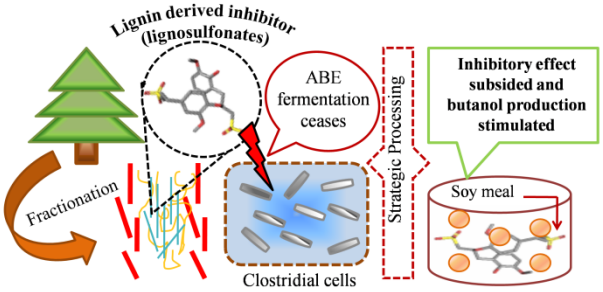
Abstract

In the present study, the effect of one of the most important lignin-derived inhibitors (lignosulfonate) was assessed. A technique to overcome the lignosulfonate inhibitory action in the acetone-butanol-ethanol (ABE) fermentation process is proposed here. Different lignosulfonates were primarily added in the fermentation medium to observe their mechanistic action on the ABE production profile. Augmenting lignosulfonate concentration (>0.5 g L^{-1}) resulted in a drastically reduced solvent titer (ABE ~1.50 g L^{-1}). Especially, low-molecular-weight lignosulfonate (>1 g L^{-1}) severely affected the solvent production and completely ceased the fermentation process. Therefore, a strategic approach that triggers the key genes responsible for butanol production was explored. The experimental analysis revealed that soy meal addition could enhance *Clostridium acetobutylicum* survival in the presence of lignosulfonates (0.25-3 g L^{-1}). Moreover, soy meal addition also enhanced butanol concentration over 1.5-fold as compared to the control experiment. The ABE production using wood hydrolysate also produced substantial solvent titer (ABE ~11 g L^{-1}) in the presence of soy meal (5 g L^{-1}). The transcriptional analysis results showed that important genes in clostridial metabolic pathways were up-regulated in the presence of soy meal addition during fermentation.

Keywords: biobutanol, clostridial fermentation, gene expression, lignosulfonates, soy meal

Graphical abstract



Introduction

Climate change has been a major concern and has devastating consequences. Besides, the escalation of carbon dioxide levels and GHG emissions have renewed interest in adopting alternative energy resources.[1] Biomass could be one of the most promising sources of renewable energy that can be converted to biofuels via thermochemical and/or biochemical routes.[2] Among different available biofuels, biobutanol has outstanding benefits to be used along with gasoline in the automotive market.[3] Butanol blends have been expected to improve fuel economies due to high energy content as like gasoline.[4] Moreover, butanol has a wide range of applications to be used as a commodity chemical.[5] Therefore, biobutanol production has been keenly studied globally and various prominent stakeholders are taking a lead to propose commercialized biobutanol in the near future.

Although biobutanol is a fascinating alternative biofuel with many benefits in the current transport fuel segment, it is still struggling to be commercialized especially because of lower production yields. High feedstock cost, dilute solvent concentration, end-product inhibition, low substrate conversion, and overall operational complexity are few identified bottlenecks in the large-scale production of biobutanol.[6,7] Interestingly, many ongoing research studies based on second-generation biomass and advanced bioprocess techniques are encouraging. Valorization of lignocellulosic biomass to biofuel also provides notable benefits to the community like (i) obliquely serving CO_2 fixation in the atmosphere, (ii) reducing air pollution from biomass incineration, and (iii) bringing energy security for oil-importing countries and many others.[8]

Lignin is a prominent polymer present in the second-generation lignocellulosic feedstock. It is a non carbohydrate biopolymer that impedes the access of the holocellulosic fraction for subsequent processes.[9] It is also lethal to many fermentative microorganisms including Clostridia. Hence, effective removal of lignin is highly desirable for successful clostridial fermentation. Several physical, physicochemical, and chemical methods have already been explored to break the hemicelluloses-lignin cross-links.[10] Lignosulfonate is one of the lignin-based organic polymers generated in the wood/paper processing industry.[11] Many softwoods and hardwoods on sulfonation produce lignosulfonates as a waste byproduct. Unlike other inhibitors (furfural and HMF), lignosulfonates are essentially lignin in sulfonated form and primarily found in softwoods (especially in spruce/pine).[12] They could hinder acetone-butanol-ethanol (ABE)

fermentation performance because of their unique properties (anionically charged watersoluble and highly acidic). It is evident from the literature that lignin and its derivatives downregulate glycolysis and fermentative pathways of Clostridia.[13] Moreover, the functional groups (especially ortho substituents) attached to the benzene ring can form an intramolecular hydrogen bond with carbonyl hydrogen. This potentially increases the cell membrane permeability and electrophilicity, thereby affecting the microbial fermentation.[14]

In the traditional Kraft and sulfite pulping process, woodchips are majorly fractionated into pulp along with the number of waste products ending up as a complex mixture in the black liquor.[15] The waste products including extractive, hemicellulose-degraded carbohydrates and lignin are burnt in a recovery boiler for energy and power generation.[16] Incidentally, the heating value of hemicelluloses is quite low and hence it can be utilized as a sugar source for value-added biochemical production. Several researchers have diversified hemicellulosic fraction (wood/black liquor) to biobutanol production.[17−19] Moreover, numerous degradation compounds derived from sugars and lignin severely limit the efficient utilization of lignocelluloses.[14] Hence, detoxification using overliming with calcium hydroxide, adsorption by activated charcoal and/or ion-exchange resins, and biological treatment are desirable.[20,21] Although these detoxification approaches are available to alleviate the inhibitory effect of degradation products, their removal efficiency differs based on processing parameters along with some sugar losses.[22] Therefore, the development of an advanced strategy that subsides inhibitor hindrance and essentially stimulates ABE production is imperative.

Clostridium acetobutylicum secreted 102 responsive proteins against butanol-challenged conditions that highlight the importance of proteins in microbial defense mechanisms.[23] Defense protein stabilizes their structure, controls amino acid metabolism and lipid metabolism, and maintains cellular integrity.[23,24] Thus, the current study hypothesizes that the fermentation performance can be enhanced by supplementing protein-rich sources (soy meal), which alleviates the effects of lignosulfonates in the fermentation medium. Soybean is a legume that is widely used for oil extraction and as animal feed. The soybean meal can have crude protein ranging from 44 to 48%, [25] and it is also an excellent amino acid source.[26]

To the best of our knowledge, the effect of lignosulfonates on ABE fermentation is not fully investigated to date. Therefore, an attempt was made to present an organized study to evaluate the effect of lignosulfonates on clostridial fermentation. Lignosulfonates were specifically assessed in varied molecular sizes to find the most lethal ones. This study also enlightens a unique strategy to get rid of the inhibition caused by the most common fermentation inhibitors from the real wood liquor. Furthermore, the molecular mechanism behind microbial tolerance was also studied to get an insight into gene expressions.

Materials and Methods

Materials: Different lignosulfonates designated as DP1566, DP1567, and DP1568 having molecular weights of 58 200, 54 000, and 6900 Da, respectively, were obtained from Borregard, Norway. Soy meal was purchased from Himedia, India, with a composition of (% on a dry basis): crude protein (50.0), total nitrogen (8.0), fat (≤1.0), and other micro/macrominerals. All other nutritional components were procured from different vendors from Finland, U.K., USA, Switzerland, and Germany. The chemicals used in the current study were of analytical grade.

Microbial Strain and Seed Inoculum Preparation: *Clostridium acetobutylicum* ATCC 824 was purchased from DSMZ, Germany (German Collection of Microorganisms and Cell Cultures). First, sporulated cells were activated by heat shock treatment at 80 °C for 10 min. The activated spores (2.5 mL) were inoculated in 125 mL airtight, anaerobic glass bottles containing 100 mL of sterile reinforced clostridial medium (RCM). The RCM contained (g L^{-1}) meat extract (10.0), peptone (5.0), yeast extract (3.0), glucose (30.0), starch (1.0), sodium chloride (5.0), sodium acetate (3.0), and Lcysteine (0.5), and the pH was adjusted to 6.8. The RCM culture bottles were incubated for 20 h at 37 °C and further used as inoculums in batch fermentation experiments.

Batch Fermentation: Batch experiments were carried out in 125 mL airtight anaerobic bottles with 100 mL of the production medium. The production medium (P2) reported by Survase et al.[27] was used in all fermentation runs unless otherwise stated. Fermentation was conducted in four different batches as (i) LS, which is lignosulfonates addition, (ii) SM and TF, which is soy meal and tofu supplementation, (iii) LS + SM, which is lignosulfonates along with 1 g L^{-1} soy meal, and (iv) the real wood hydrolysate supplemented with soy meal. In the first batch, three different lignosulfonates, namely, DP1566, DP1567, and DP1568 were individually added (at varied concentration ranges of 0.25-3 g L^{-1}) in the P2 medium. Alternatively, Tofu and soy meals were also supplemented separately in a second set of fermentation to observe their effect on ABE fermentation. Further, lignosulfonates and soy meal were included together (at varied concentrations) to test their synergistic action on solvent production.

In the last fermentation set, the real wood (spruce chips) liquor was used as a carbon source in the presence of soy meal. Conditioning of wood liquor was carried out prior to the fermentation experiments as per the method reported by Sklavounos et al.[28] to avoid fermentation inhibition. Spruce chips were fractionated in a silicon oil bath at 150 °C for 30 min. The resulted pulp was manually squeezed and washed to release diluted SO_2-ethanol-water (SEW) spent liquor. This liquor was then subjected to vacuum evaporation followed by centrifugation and steam stripping to remove colloidal precipitate and SO_2, respectively. The steam stripped liquor was limed with $Ca(OH)_2$ to pH 9.0 and low-molecular-weight chitosan (0.5 g L^{-1}) was added to it. Thereafter, the precipitate was removed by centrifugation and washed three times with alkaline water. Besides, sulfite anions in the liquor were oxidized to sulfate anions by air bubbling in the presence of $FeSO_4 \cdot 7H_2O$ catalyst to get the CATOX liquor. Finally, the conditioned CATOX liquor was subjected to nanofiltration, and the resultant permeate was diluted 4-fold to reduce the inhibitor concentrations below the lethal level (1 g L^{-1}). Furthermore, the liquor was supplemented with pure glucose as required to reach the final fermentation sugar concentration of 60 g L^{-1} for better comparison with other experiments. It should be noted that all of the supplementations were done in the beginning and pH was adjusted to 6.5, if necessary. The fermentation medium was then purged with nitrogen, autoclaved at 121 °C for 20 min, and cooled. Fermentation was initiated by inoculating 5% (v/v), 20 h old inoculum and incubated at 37 °C for 96 h. The P2 medium was used as a control. The samples were withdrawn after 96 h for subsequent solvent and residual sugar analysis.

Analytical Methods: The solvents (ABE) and acids (acetic and butyric acid) were quantified as described by Survase et al.[18] Gas chromatography (Hewlett-Packard series 6890) equipped with a flame ionization detector and DB-WAXetr capillary column (30 m × 0.32 mm × 1 μm) was used. The injector temperature was kept at 200 °C and detector temperature at 250 °C. The 10 μL

of the sample was injected for analysis. Sugars were determined by high-performance liquid chromatography (Bio-Rad Laboratories, Richmond, CA) equipped with an Inores S 259-H column (Inovex, Vienna, Austria) packed with Inores cation exchanger (particle size, 9 mm). The column was heated at 70 °C, and the eluent (0.01 M H_2SO_4) was circulated with a flow rate of 0.60 mL min^{-1}. Cellobiose (Roth, Karlsruhe, Germany) solution was used as an internal standard and a refractive index detector (model 1755; Bio-Rad) for quantification.

Gene Expression Protocol: Total bacterial RNA was extracted using RNeasy protect Kit (Qiagen) and further purified as per the method described in the kit manual. Before processing, the RNA samples were treated with RNase-free DNase I to eliminate genomic DNA contamination. Complementary DNA (cDNA) was synthesized using a Maxima First Strand cDNA Synthesis kit (Fermentas). Then, cDNA products were amplified by real-time fluorescence quantitative PCR with primers of ctfAB-F (5′-CAGAAAACGGAATAGTTGGAATG- 3′), ctfAB-R (5′-TGACCACCACGGATTAGTGAA-3′), adhE-F (5′-GTTTTGGCTATGTATGAGGCTGA-3′), adhE-R (5′-CAAGCGTGAAAGAAGGTGGTAT-3′), bdhB-F (5′ - ACGCTTCTGCCATTCTATCC-3′), and bdhB-R (5′-ATTGCGGCACATCCAGATA- 3′) using Thermo Scientific Maxima SYBR Green qPCR Master Mix (Thermo Scientific).[29] The 16S rRNA was used as an internal control for quantification. Real-time RT-PCR was carried out using CFX96 Real-Time PCR Detection System (Bio- Rad Laboratories, Inc., Richmond, CA). PCR conditions were as follows: an initial denaturation step at 95 °C for 10 min, followed by repeating an amplification and quantification program for 40 cycles (95 °C for 10 s, 60 °C for 60 s with a single fluorescence measurement), and a melting curve program (a continuous fluorescence measurement raising temperature from 60 to 95 °C with a slow heating unit).

Results and discussion

Effect of Lignosulfonates on ABE Production: Three different lignosulfonates (DP1566, DP1567, and DP1568) with varied molecular sizes were individually supplemented in the concentration range of 0.25-3 g L^{-1}. DP1566 and DP1567 correspond to high-molecular-weight lignosulfonate, while DP1568 is a low-molecular-weight lignosulfonate. Figure 1 depicts the ABE production profile under varied lignosulfonate concentrations. It was observed that lignosulfonate severely obstructed the biobutanol production, which was confirmed by the control experiment.

P2 (control) was able to produce butanol up to 5.20 g L^{-1} with a total ABE of 8.98 g L^{-1}. However, all three lignosulfonates were found to be detrimental at certain increasing concentrations up to 3 g L^{-1} (Figure 1). Further increase in lignosulfonate concentration (3 g L^{-1}) suddenly stopped the bacterial growth and interrupted the fermentation. A recent report by Chen and Zeng[30] demonstrated that the low concentration of lignin-derived compounds inhibited 25% butanol production, while complete inhibition was observed at a concentration of >4 g L^{-1}. They showed that the inhibition was mainly because of the reduced cell growth and the blocked acid assimilation pathway. It seems that the low-molecular-weight lignosulfonates were more detrimental to fermentative Clostridia than the high-molecular- weight lignosulfonates at lower concentrations. When the lignosulfonate concentrations were increased beyond 1 g L^{-1}, they severely inhibited the fermentation operation irrespective of the molecular weight (Figure 1).

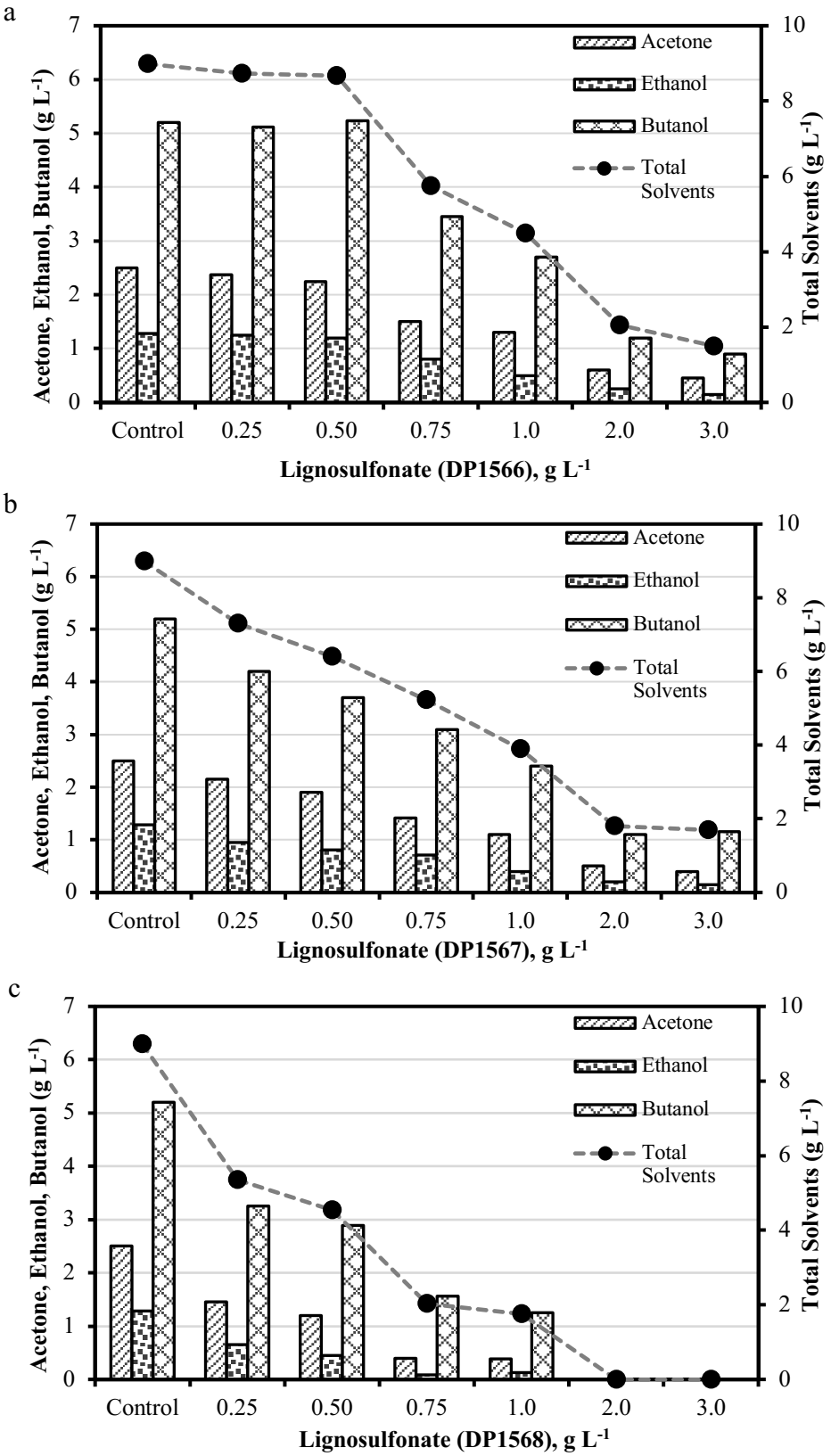


Figure 1. Effect of lignosulfonates on the ABE production with *C. acetobutylicum* ATCC 824.

Mussatto and Roberto[31] reviewed that the phenolic derivatives having lower molecular weights are more toxic. The mechanistic effects of these inhibitors are due to the loss of membrane integrity, increased proton influx, slow microbial growth, and declined sugar consumption rate.[32] Another study by Luo et al.[33] examined the physiology of *C. acetobutylicum* ATCC 824 under phenolic acids/aldehydes stress. They revealed that phenolics altered the gene expression profiles related to membrane transporters (ATP-binding cassette and phosphotransferase system). This might result in reduced glucose consumption, thereby inhibiting solvent synthesis. Besides, sporulation also ceases as histidine kinase encoding genes downregulated due to the presence of fermentation inhibitors.[33]

Stimulatory Effect of Soy Meal: Preliminary studies with soy meal and Tofu were carried out to observe their role in ABE fermentation. Interestingly, both of them significantly affected butanol production and resulted in improved solvent titer. Tofu addition in the ABE fermentation media was effective at higher concentrations (15%), while 1-5 g L^{-1} of soy meal supplementation was sufficient to reach comparable butanol titer (data not shown). Hence, soy meal was further considered in subsequent experiments. Soy meal (1 g L^{-1}) along with lignosulfonates (DP1566, DP1567, and DP1568) was fed to clostridial fermentation in the range of 1-3 g L^{-1} to check their overall inhibitory effect on biobutanol production. Surprisingly, the inhibitory effect of lignosulfonates was considerably reduced in the presence of soy meal with enhanced ABE titer (Figure 2).

Figure 2 demonstrates the solvent production profiles in the presence of different lignosulfonates together with 1 g L^{-1} soy meal (stimulator). It should be noted that all data points presented in Figure 2 were recorded after 48 h. Although soy meal greatly elevated the ABE production, it has varying impacts on the ABE performance depending on the molecular sizes of lignosulfonates. The subsidiary effect of soy meal was significant when high-molecular-weight lignosulfonates were added (Figure 2a,b). On the other hand, higher concentrations of low-molecular-weight lignosulfonates (>1 g L^{-1}) still hindered the ABE production (ABE 1.32 g L^{-1}) even in the presence of 1 g L^{-1} soy meal (Figure 2c). Therefore, higher soy meal amounts (2-3 g L^{-1}) were necessary to subside the inhibitory effect of the low-molecular-weight lignosulfonate (data not shown).

Generally, it has been seen that acid/alkali-treated liquor takes a longer time to produce ABE, which, in turn, affects the productivity. Jin et al.[34] fermented apple pomace hydrolysate and observed a low fermentation rate mainly due to the presence of inhibitors that inhibited ABE fermentation. Interestingly, the clostridial fermentation time was reduced from 96 to 48 h with the same production of ABE in the current study. This suggests that the soy meal addition is beneficial in reversing the inhibitory effect of lignosulfonates with a speedy fermentation rate.

It is known that the addition of several nitrogen sources in the production media could improve the ABE fermentation and shorten the fermentation time. One of the studies demonstrated that the addition of 3 g L^{-1} ammonium acetate contributed to improved solvent production within a shorter fermentation span.[35] In the present study, added soy meal may act as a sole nitrogen source and thus be responsible for reduced fermentation time.

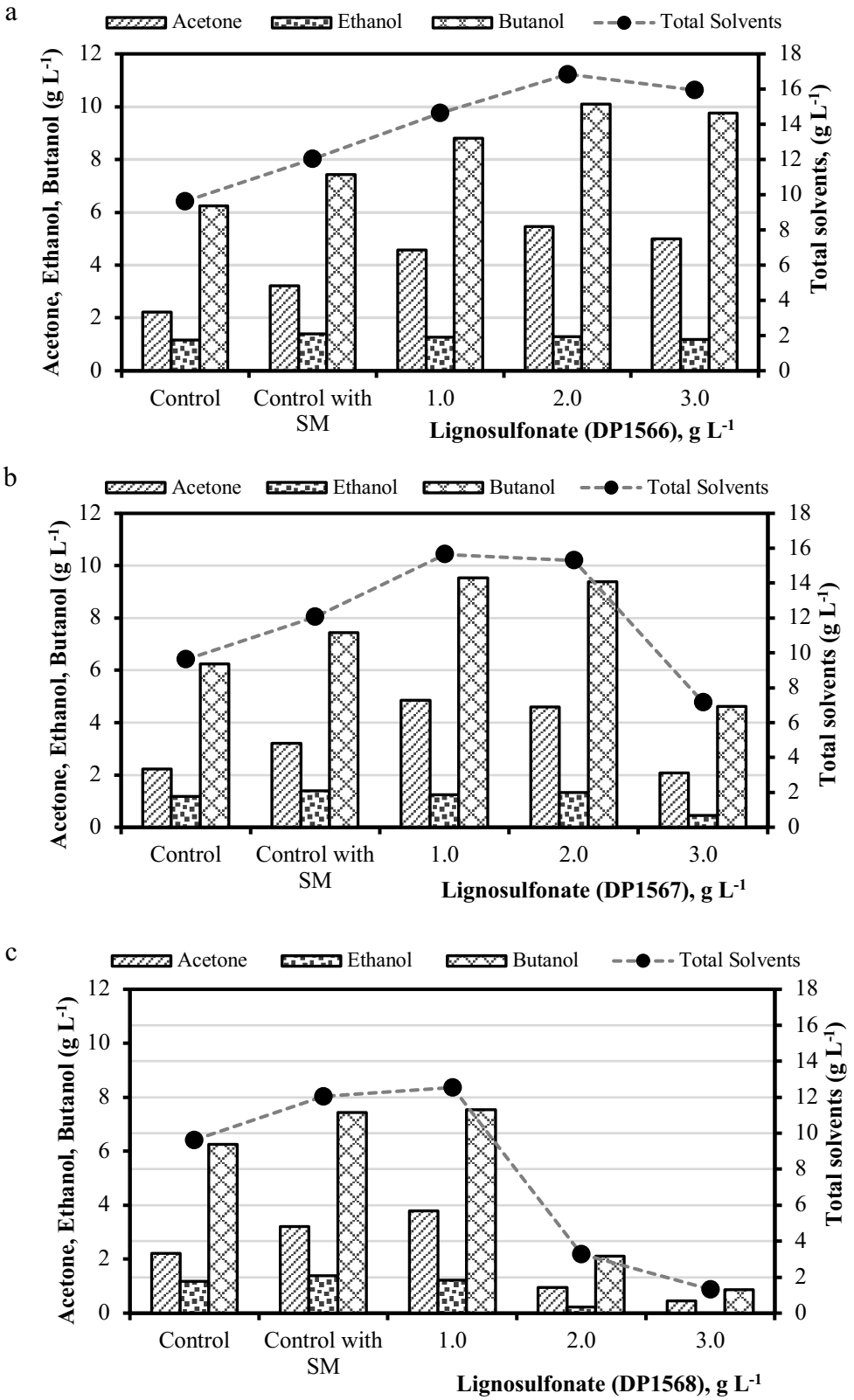


Figure 2. ABE production profiles in the presence of an inhibitor (lignosulfonate) and a stimulator (soy meal) (lignosulfonate concentration varied in the range of 1-3 g L^{-1}; soy meal concentration kept at 1 g L^{-1}).

The improvements by soy meal addition can be attributed to one of the following mechanisms:
(i) unspecific adsorption of lignin molecules on exogenous protein supplements
(ii) protective effect due to the presence of anabolic precursors (amino acids)
(iii) triggering the genes responsible for acid assimilation and solvent synthesis
(iv) peroxidase present in soy meal might help in lignin polymerization
It has been observed that soy meal particularly contains few essential amino acids, *viz*., lysine, tryptophan, arginine, and cysteine.[36] It is well reported that lysine favors both *C. acetobutylicum* survival and butanol synthesis under stressed conditions.[37] Besides, tryptophan and arginine also have a positive role in ABE fermentation. Our recent study described that tryptophan drives more metabolic flux toward butanol, while arginine could be useful in energy (ATP) build up.[38] Moreover, it has been demonstrated that pelleting soybean meal with calcium lignosulfonate protects protein degradation in the rumen.[39] Similarly, lignosulfonates might be coupled with added soy meal and result in a lowered inhibitory effect in the present investigation. Overall, soy meal addition served a dual purpose: (i) fermentation performance was raised by 70-90% even in the presence of lignin-derived inhibitors and (ii) butanol production was specifically upregulated within a shorter fermentation span. These findings suggest that soy meal acted as a detoxifying agent and stimulator during ABE fermentation.

The positive effect of soy meal on ABE performance instigated us to observe their protective impact on the real wood liquor obtained by SEW fractionation of spruce chips. SEW fractionation process was developed at Aalto University, Finland, to fractionate different lignocellulosics[28] and the resultant SEW spent liquor was used in the present study. The composition of wood liquor is tabulated in Table 1. Wood liquor was conditioned to remove many inhibitory elements and used as a carbon source in ABE fermentation. Soy meals of 1, 3, and 5 g L^{-1} were added to wood liquor to check if it subsides the inhibitory effect of lignin or not.

Table 1 Composition of the Real Wood Liquor

	Values (g L^{-1})	
particulars	before conditioning	after conditioning[a]
formic acid	2.2	NM
acetic acid	0.3	NM
methanol	0.2	NM
ethanol	2.6	NM
5-HMF	0.4	NM
furfural	0.6	NM
lignin	60.0	24.6
glucose	27.9	23.2
xylose	22.3	19.2
galactose	10.1	11.8
arabinose	5.6	6.4
mannose	40.5	44.3

[a]NM- not measured

Figure 3 describes the ABE production profile from wood liquor in the presence of soy meal. It is seen that wood liquor is successfully consumed by Clostridia to produce 10.9 g L^{-1} ABE when supplemented with 5 g L^{-1} soy meal. It has been reported that the supplementation of micronutrients/cofactors at low concentrations could maintain high ORP (oxidoreduction potential) levels, which is directly attributed to the earlier initiation of solventogenesis with improved butanol titer.[40] Moreover, several research studies also pointed out that a higher concentration of metabolic precursors is detrimental to clostridial butanol production.[41,42] Hence, soy meal addition was not continued beyond 5 g L^{-1} in the present study. Nevertheless, around a threefold increment in butanol titer was achieved as compared to control (without soy meal). This outcome proves that soy meal can strongly subside the inhibitory effect of wood liquor (especially lignin) during clostridial fermentation. A recent study showed that $CaCO_3$ supplementation can also alleviate the inhibitory effect of lignocellulose-derived weak acids on ABE production.[43] Furthermore, a transcriptional analysis was carried out to understand the molecular mechanism behind the solvent improvement, which is discussed in the subsequent section.

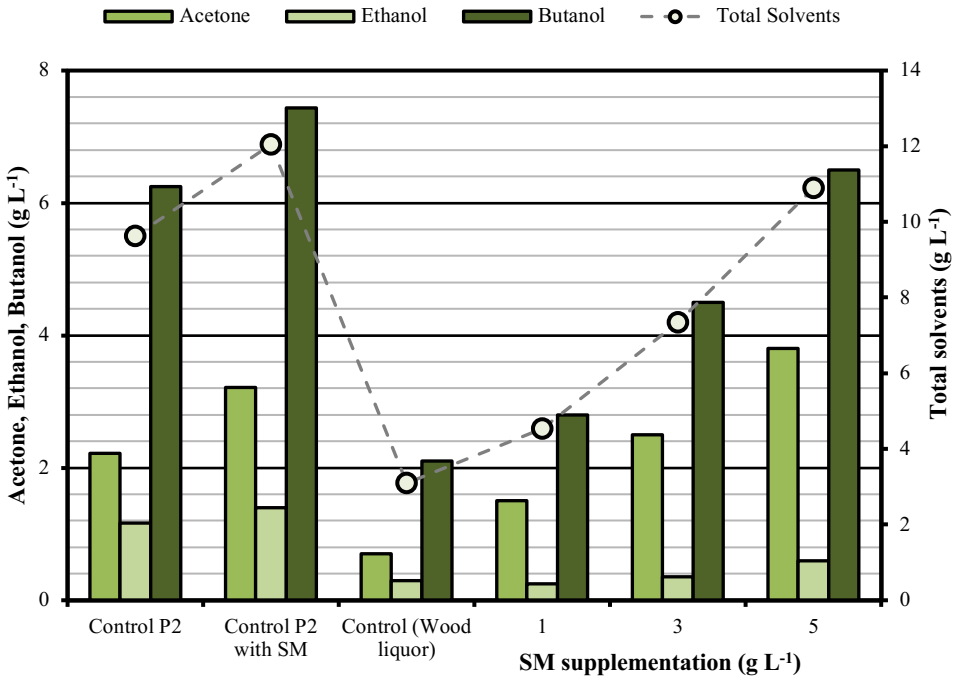


Figure 3. ABE production from wood liquor supplemented with soy meal in the concentration range of 1-5 g L^{-1}. SM, soy meal.

Effect of Soy Meal Supplementation on Genetic Transcription Level: ABE fermentation involves an acidogenic phase concomitant with exponential biomass growth and the carboxylic acid formation and thereby solvent production toward the stationary phase (Figure 4). As we approach the end of fermentation, sporogenesis and cell autolysis occur simultaneously with the accumulation of cytotoxic solvent to a strong inhibitory level.[44] The redox shuttles NAD(H) and NADP(H) are known as biomarkers of microbial metabolic activity with NAD(P)H-dependent enzymes playing a key role in butanol biosynthesis.[45] Most of the physiological and biochemical processes take place through the redox reactions in microorganisms. Therefore, proper redox balance is essential for maintaining normal cell growth and physiological metabolism.[46]

Interestingly, NADH plays an important role in maintaining the redox balance and also affects product formation along with metabolic flux redistribution.[47]

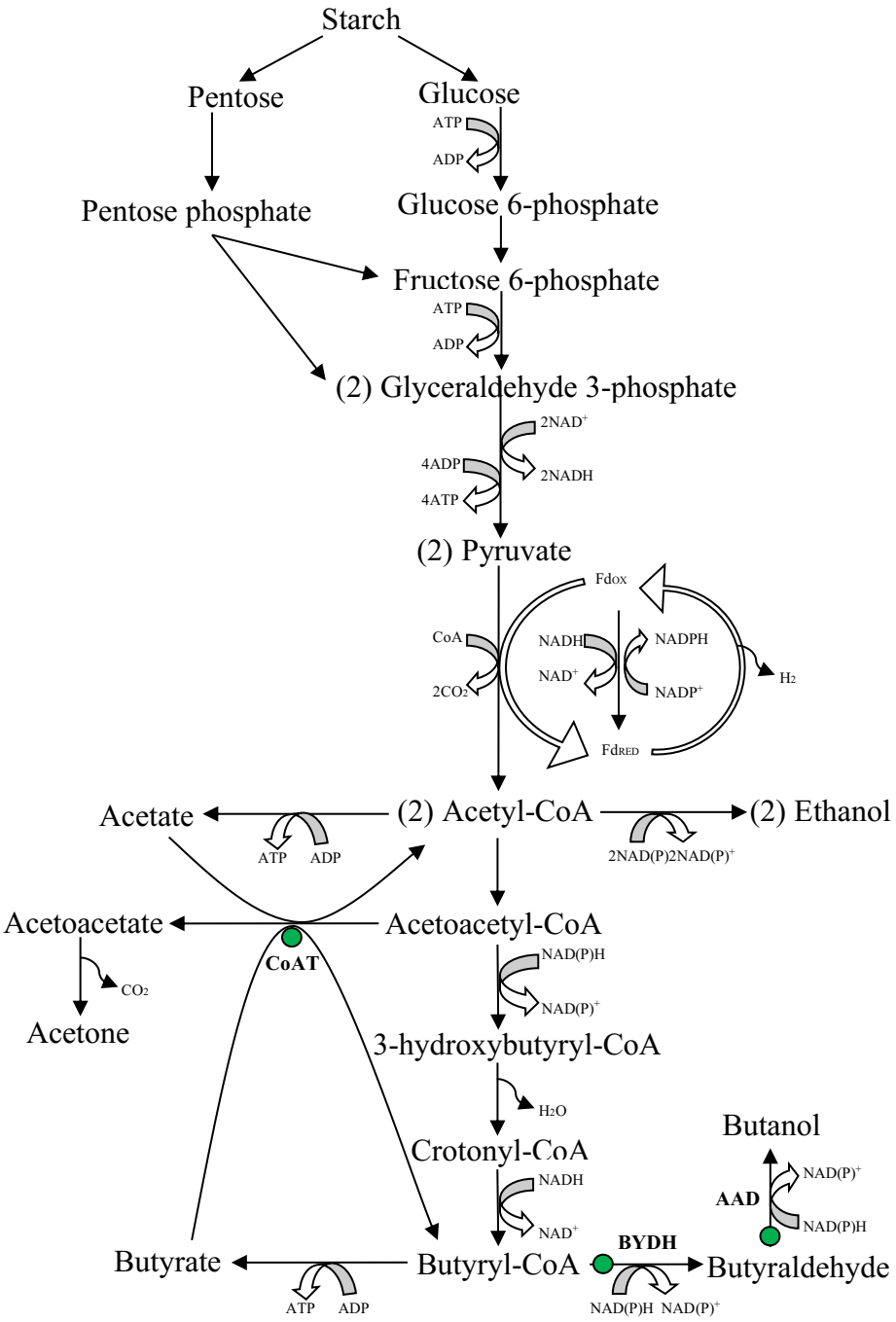
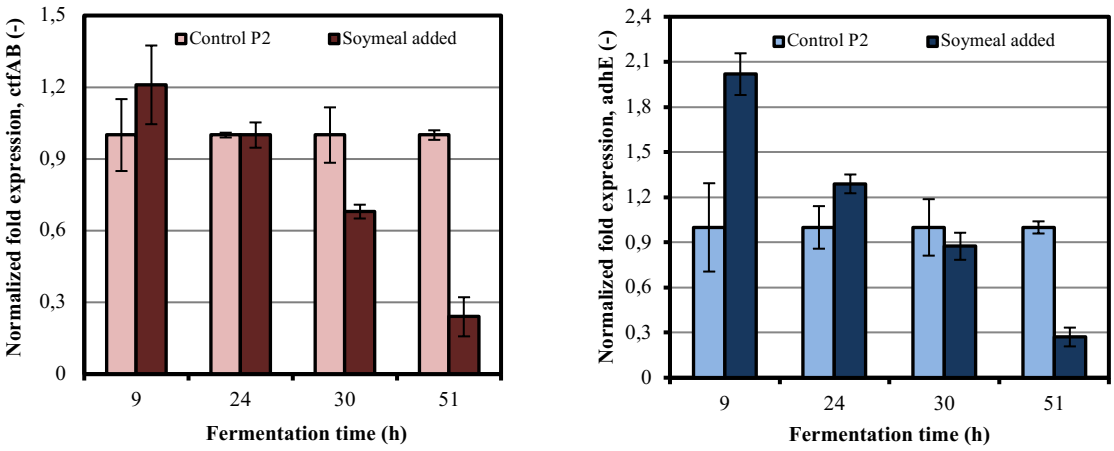


Figure 4. Metabolic pathway of butanol biosynthesis by *C. acetobutylicum* ATCC 824.

Previous studies have evaluated that butanol production could be significantly improved by elevating intracellular NADH levels through overexpression and/or knocking out key genes in metabolic pathways.[48,49] In the current study, changes in genetic transcriptional levels of the key

enzymes responsible for the ABE production were analyzed to find how soy meal addition prompts phase shift and improves butanol titer. Generally, it has been demonstrated that CoA-transferase (encoded by ctfAB gene) is solely responsible for the phase transition from acidogenesis to solventogenesis and also reassimilate the produced organic acids.[50] On the other hand, butyraldehyde dehydrogenase (encoded by adhE gene) and butanol dehydrogenase (encoded by bdhB gene) are the two enzymes directly linked to butanol biosynthesis.[51] Hence, the transcriptional levels of these three genes were quantified over the fermentation time intervals by PCR measurements.

The normalized fold expressions and/or fold change were estimated by the ΔΔCt method and further used to interpret the outcomes. Figure 5 shows the transcriptional profiles of ctfAB, adhE, and bdhB genes. Interestingly, the expression levels of all three genes were significantly increased when soy meal was supplemented in the production medium. Particularly, the adhE gene showed the highest fold expression (2.01), which, in turn, activated butyraldehyde dehydrogenase enzyme, leading to improved butanol biosynthesis. Also, it was observed that these key genes were specifically upregulated in the early stages of fermentation (9 h), and thereafter a gradual decline in expression levels was noted (Figure 5). Especially, the early onset of the ctfAB expression led to the CoAtransferase activation, which eventually promoted phase shift and accelerated organic acid reassimilation. These could be one of the possible reasons behind the faster fermentation rate observed in soy-meal-supplemented runs.



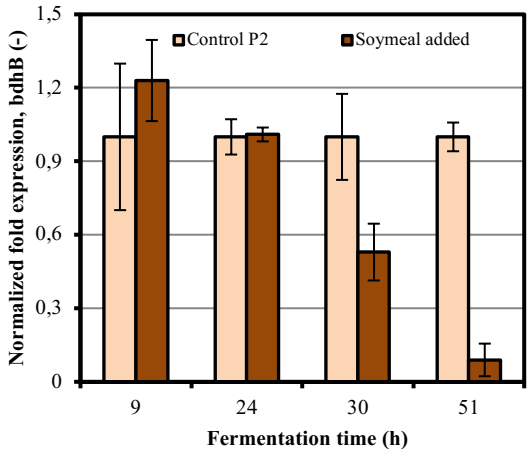


Figure 5. Variations in transcriptional levels of ctfAB, adhE, and bdhB with or without soy meal supplementation in ABE fermentation.

Overall findings from this study prove the fact that precursors/stimulators supplemented in the production media play a crucial role in regulating gene expressions and in turn biological functions of many metalloenzymes.

Conclusions

The effect of lignosulfonates formed during sulfite processing of softwood/hardwood on butanol production using *C. acetobutylicum* ATCC 824 was investigated. The fermentation profiles showed that the low-molecular-weight lignosulfonate had a stronger inhibitory effect on cell growth and solvent synthesis as compared to high-molecular-weight lignosulfonate. Final butanol and the total ABE titers under low-molecular weight lignosulfonate (1 g L^{-1}) were 1.25 and 1.76 g L^{-1}, which were reduced by 76 and 80%, respectively, in comparison with control. Hence, soy meal, a protein-rich supplement was added in the P2 medium to stimulate Butanol production. As a result, butanol and ABE concentration reached 7.54 and 12.55 g L^{-1}, respectively, even in the presence of 1 g L^{-1} lignosulfonate (low molecular weight). This improvement was attributed to the stimulatory action of soy meal, which was supplemented at 1 g L^{-1} concentration. However, it was observed that a higher soy meal concentration (5 g L^{-1}) was needed to prevent the inhibitory effect in the real wood hydrolysate. Subsequently, transcriptional analysis of *C. acetobutylicum* in the presence of lignosulfonate together with soy meal was investigated. The results indicated that soy meal upregulated expression profiles of genes related to key enzymes, *viz*., butyraldehyde dehydrogenase, CoA transferase, and butanol dehydrogenase. This approach avoids the inclusion of other harsh detoxification techniques, which often lead to sugar losses at every step. Hence, the present technique efficiently produces biobutanol from the second- generation feedstocks without sacrificing the fermentable sugars during a multistep detoxification approach. Overall, the proposed strategy could be useful in alleviating the inhibitory effect of several microbial inhibitors and simultaneously improving the final solvent titer.

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Notes
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