Research Article

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Comparison of Saccharification And Lactic Acid Production Efficiencies From Chemically and Biologically Pretreated Wheat Straw

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Abstract

Background

In recent times, agricultural waste has been utilized in many industries as an energy source in several processes. Wheat straw, one of the most cost-effective raw material, has a great potential to be utilized as a substrate in fermentation to produce lactic acid. Utilization of agro waste material as the carbon source provides a cheap and environment friendly production of lactic acid.

Methods

A comparison of chemical and biological pretreatment of wheat straw and the use of pretreated wheat straw hydrolysate in fermentation of Lactobacillus was carried out using optimized process parameters of temperature and ph. Pretreatment methods using dilute acid and white rot fungus Coriolis versicolor were performed on wheat straw as well as the effect of co-pretreatment with calcium hydroxide on differentially pretreated wheat straw was studied by analyzing sugar and lactic acid concentrations during fermentation. The effect of different pH (7, 8, and 9) on fermentation using dilute acid pretreated wheat straw was observed to lower the lactic production with the increase in ph. Dilute acid pretreatment of wheat straw provided the highest concentration of glucose at 57.2mg/L and subsequently enhanced yield of lactic acid during fermentation while the biologically pretreated wheat straw hydrolysate provided comparatively lower concentration of glucose at 49.2mg/L and thus decreased lactic acid production.

Result

The final lactic acid concentration of 48.14 mg/L was obtained in dilute acid pretreated wheat straw with Yp/s of 0.915 mg/mg, while the use of biologically pretreated wheat straw hydrolysate provided 39.56 mg/L concentration of lactic acid and an Yp/s of 0.908 mg/mg. However, a reduced lactic acid concentration of 27.03 mg/L and 20.62 mg/L was seen after the co-pretreatment of both dilute acid and fungal pretreated wheat straw with calcium hydroxide respectively, indicating its adverse effects on fermentation process.

Conclusion

Pretreatment of wheat straw with dilute acid can be considered a good saccharification method to obtain the optimum concentration of lactic acid in microbial fermentation carried out at optimum temperature and pH conditions.

Keywords: Lactic Acid, Solid State Fermentation, Pretreatment ,Wheat Straw Agro-Residues, Isolated Bacteria, Coriolis Versicolor

Introduction

Demand for production of various chemicals in industry keep rising to fulfil human requirements that grow over time, thus greater amounts of lactic acid are produced each year through fermentation to meet various industrial needs such as, food preservation, pharmaceutical drug production and in leather industry[1] . Because of the great industrial significance, D- Lactic acid is produced as an intermediate in the synthesis of chiral material, lactic acid does not only act as an effective raw material but is also responsible for improving the quality and efficiency of poly lactic acid,which is the most important value added chemical that plays role in various industrial and research areas [2].

In biotechnology different methods and experiments are applied to obtain the desired and valuable results and products, Fermentation is one of the primitive and sophisticated methodology that is currently being used, generally linked with food processing and preservation[3].

It can be assumed that agricultural waste or by-products such as wheat straws are low-cost materials because they are available in nature, relatively cheap, necessarily involve little handling, and are effective materials. According to their physicochemical properties and low cost these materials are available in large quantities and have the potential to produce useful and beneficial product. As a strategy for diversifying energy resources, stimulating rural economic development and minimizing greenhouse gas emissions, biofuel production from agricultural waste is currently attracting global attention. Ethanol is an important solution transport fuel to oil for automobiles with modified engines or as an additive in fuel blends to improve engine performance. Pretreatment to disrupt the structure of the rigid cell wall, enzymatic hydrolysis to grind the polysaccharides and subsequent fermentation are major processes for producing ethanol from lignocellulosic biomes. Due to low fermentation efficiency and ethanol titters, high enzyme cost and high-water consumption, the commercialization of second-generation bioethanol from lignocellulosic biomass is still under development. Advanced technology is imperative to achieve high yields of ethanol and titter ethanol with low enzyme loads. Different pretreatment techniques were employed to improve enzyme digestibility. Lactic acid is an important and useful industrial chemical used in food industry as an oxidizing agent and preservative, as well as other pharmaceutical applications. Many companies have recently expressed interest in manufacturing lactic acid for plastics that are biodegradable. This has resulted in extensive screening of various possible substrates for lactic acid overproduction to meet the future demand[4].

The main concern of industrial lactic acid fermentation is to minimize the material cost and improve the performance and efficiency of the process.Several renewable materials, such as wheat straw and corn Stover have been previously used for the value-added processing of lactic acid[5]. A major problem associated with fermentation is the pretreatment of lignocellulose with high cost enzymes and materials. Biological or chemical pretreatment of lignocellulose thus comes as one of the cheap methods for chemical production. Therefore, use of cheap materials and substrates for lactic acid production would effectively reduce the overall production cost and thus having a positive impact on many other industrial areas where it is being used.

Wheat Straw

When the wheat grains are harvested the resulting raw material is wheat straw, therefore, this raw material is extremely value able, capable of feeding animals in one way and creating desirable items such as lactic acid, laccase and other. The comprehensive characterization of wheat straw is necessary and useful for producing new products with known properties[6].The production of wheat straw has recently increased, wheat is a beneficial approach for the production of bioethanol. Modern extraction technologies has been improved to remove the value added chemical from wheat straw to enhance the profitability of industrial products. The burning of wheat straw in greater quantities is an open invitation for global warming and carbon dioxide emission, to overcome this problem, fermentation of wheat straw for desire products is an encouraging approach to reduce the pollution and improve the economy[7]. Wheat straw is an extensive source of lignocellulose, Wheat straw contain cellulose, hemicellulose, lignin and protein which is a cheap source for chemical production[8].

Wheat Straw As Agriculture Waste

When agro-industrial residues are known, these residues are the method for the processing of various useful chemicals such as lactic acid, ethanol, biofuel, biogas, and mostly used in different areas of research. This promising approach is highly feasible to mitigate the rate of emissions and reduce costs [9].

Wheat straw is one of the cheapest and most attractive source of fermentation material in the world, as it typically contains about 70% carbohydrate and can therefore be used by microbial fermentation. In the processing of industrial activates, agro-industrial residues and waste are generated; in addition, substances such as straw, plant, stock, leaves, etc[10]. In recent decades, wheat straw, an important and qualitative source of biomass, has frequently been considered a feasible option for material production. Preferably, this waste is now attracting the researchers due to its efficiency for industrial work[11]. In order to achieve the maximum production rate it is important to know that all parts of the straw are not significant for industrial utilization [12].

What is Lignocellulose?

Lignocellulosic products, like wood, agricultural or forestry waste, are a mixture of natural polymers based on lignin, cellulose and hemicellulose, and tannins with more than two hydroxyl groups per molecule. Lignocellulose is one of the cheapest and most renewable sources of fossil fuel replacement. Structural, cellulose appears to be linked to several mixtures of plants and can influence their biodegradation.Cellulose is the vital ingredient of lignocellulose accompanied by lignin and hemicellulose. Cellulose and hemicellulose differ in the sense that hemicellulose has branches with thin lateral chains covering multiple sugars and oligomers that can be easily hydrolyzed [13]. The lignocellulose disposal and byproducts of agro-industrial activities can be divided into a few groups; those in which, in addition to the output of lignocellulose residue, there is a market for fruit processing and those in which the lignocellulose source is the important source comprising agro-industrial waste from the processing of wheat and wood and the accumulation of simple carbohydrates [14].

Under normal circumstances and climate, lignin would not be able to degrade, but it has an important issue that restricts the use of lignocellulose for various purposes (Zhu et al., 2011:1029). The concern of non-degradability has been made simpler by the use of microorganisms such as bacteria that generate various natural enzymes to physiologically breakdown lignocellulose[15].

Lignocellulose as a rigid, lattice structure of lignin-protected polysaccharide microfibers, it protects polysaccharides from interference of hydrolytic enzymes and external factors and helps to sustain the compound's complex structure. The polysaccharides most frequently formed include the microfibers of hemicellulose and cellulose filaments[16].

Table 1. 1: The composition of lignocelluloses substance (Eika W. Qian, 2014)[16]

Straw	Lignin %	Hemicellulose%	Cellulose %
Wheat	16-21	26-32	29-35

Figure 1. 1: Collection of Wheat StrawCoriolisVersicolor for Pretreatment

Pretreatment of agriculture waste is an important process for obtaining the sugar and value-added chemicals. This white rot fungus is a type of mushroom which contributes in many medicine preparations. Therefore, it has the potential to degrade the lignin and providing the environment Wheat straw was used as the substrate for the production of Lactic acid. The straw was collected by conventional harvest equipment from a commercial farmers' field in Northern Cyprus. The collected straw was stored in black polyethylene bags and stored at room temperature (25 oC) and ambient relative humidity. Prior to the fungal and acidic pretreatment, the wheat straw sample were washed with distilled water in other to get rid of the dust and other contaminants to certain level. It was then oven dried at 60 oC for 48 hours. The dried wheat straw was milled with a blending machine. Total weight of the blended wheat straw is 500 g.

Solution Preparation

During the entire experiment, different solution and buffer have been prepared according to the requirement and conditions.

Buffer Solutions

Buffer solution were prepared, 3.9 g of 0.1M sodium dihydrogen phosphate dissolved in 250ml of distilled water and 0.7 g of 0.1M disodium hydrogen phosphate dissolved in 50 ml of distilled water by adding the few drops of base on acid, adjust the pH at 6 by pH meter, get the buffer to be used for experiment.

Pretreatment of Wheat Straw

Lignocellulosic biomass is a potential source for production of chemicals, biofuels and organic acids. Due to complex structure of lignocellulosic biomass it can't be used directly therefore fermentation is required. Pretreatment plays an important role in separating cellulosic material from lignin fractions i.e. it breaks down the lignin barrier leading to release of cellulosic content (Anwar et al., 2014) which further breaks down upon pretreatment leading to release of glucose, xylose, xylanose, arabinose which upon fermentation gets converted to product. 2 Pretreatment method were used for pretreating the wheat straw and they include: (a) Dilute acid pretreatment and (b) biological pretreatment.

Dilute Acid-Based Pretreatment Method

10g of the Wheat straw sample was weighed and mixed with dilute sulfuric acid (4 % v/v). The mixture was adjusted to pH 5 using 10M NaOH and then placed inside the autoclave at 121 C for 1 hour. After autoclaving, the mixture was filtered and the filtrate were centrifuge at 1500 rpm for 5 minutes, the supernatant were evaluated for its sugar concentration by phenol sulfuric method.

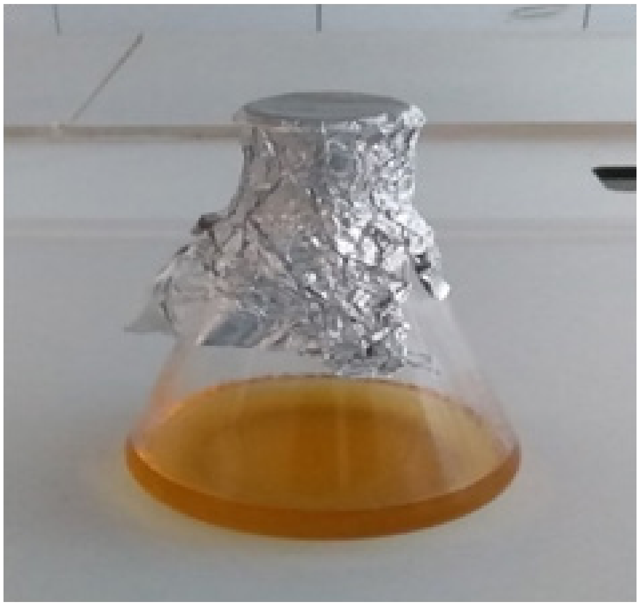

Figure 2.1: PretreatedWheat Straw by Dilute Acid

Biological (Fungal) Pretreatment

60 g of wheat straw sample were weighed and humidified by buffer solution, flask was covered by foil and kept it in autoclave for sterilization, after cooling the sample were inoculated with the selected fungi Coriolis versicolor that was already available in the microbiology laboratory. The culture was incubated at 30 C for 21 days. After the incubation period, the culture was dried in an oven at 40 oC for and then blended to fine powder.

The pretreated substrate was kept in buffer solution for 20 minutes, suspended solid (1g of sample into 10ml of buffer) and centrifuge at 1200 rpm for 15 minutes, the crude filtrate were analyzed for its sugar concentration by phenol sulfuric method.

Figure 2. 2: Pretreatment Of Heat Straw By Coriolus Versicolor

Phenol Method For Determination Of Glucose Content In Pretreated Samples

100mg/mL Glucose solution was prepared and used to make different standard solutions of 10, 20, 40, 60, 80 and 100 mg/mL. 0.2 ml of 5 % phenol was added into each conc. of glucose standard test tubes as well as the tubes containing pretreated sample (both the acid and fungi pretreated sample) followed by the addition of 1 ml 4 % (v/v) sulfuric acid into all test tubes. The content in each tube (Glucose standard solution and Sample) were properly mixed and the absorbance were determined at 490 nm in a spectrophotometer. The leaner equation as presented in Figure 2.1 were used to determine the glucose concentration of the samples.

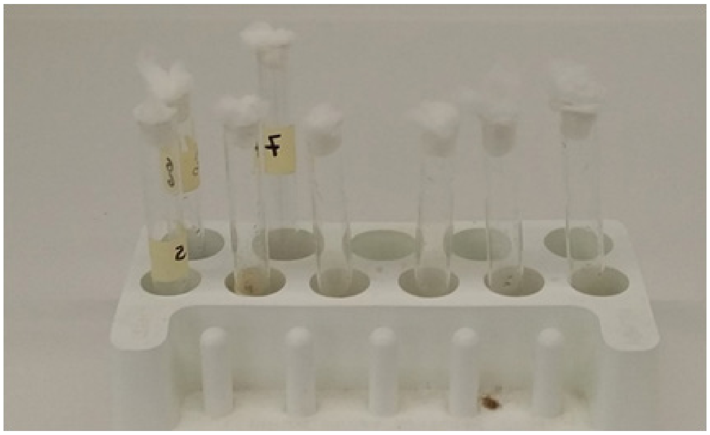

Figure 2.3: Standard Solution Of Glucose And Sample

Preparation Of Inoculum

Mixed bacteria culture isolated from diary waste were used for as the inoculum. Briefly, the dairy waste was mixed with water and filtered, the filtrate was place 20 ml of nutrient broth and incubator at 37 C for 24 hours. After 24 hours bacterial growth wasobserved, and the bacteria culture were used for the fermentation of pretreated and processed wheat straw for Lactic acid production.

Fermentation Experiment

This experiment was performed in 500 ml flask, 100 ml of pre-treated wheat straw hydrolysate were used as substrate, 4M KOH were used to maintain the medium at pH 6 and at a temperature of 35.5 oC culture mixture were set at agitation rate of 150 rpm, the microbial inoculum in the medium was 10 % v/v in both flask with or without the presence of Ca (OH) 2 or without over liming. The concentration of lactic acid was measured at different time intervals in both cases.

Determination Of Lactic Acid Concentration

The concentration of lactic was measured by spectrophotometer in which, the fermented liquid sample was measured by making 20-fold dilution. 1.2 g dissolve 100 ml in H20 the known concentration of lactic acid then series is prepared using 2 times dilution and measured the absorbance of all test tube including sample at 390 nm in spectrophotometer.

Sterilization

Throughout the process, Sterilization is very crucial in each experiment to prevent the contamination, therefore, all the containers and solution were covered with aluminum foil and heated at 121 C at least for 15 minutes. Safety cabin and ethanol were used to remove the contamination from glass and plastic test tubes.

Figure 2.4: Sterilizing The Material And Solution

Results And Discussions

In this section the experiment results are discussed, along with glucose measurements for the different types of Wheat straw pre-treatment methods (Acid and fungi). The results of lactic acid yield for different types of pretreatment methods are shown, as well as lactic acid production at different pH were observed.

Effect Of Different Pretreatment Methods On Glucose Concentration In Wheat Straw

The effect of different pre-treatment methods notably Acidic and fungal treatment on glucose concentration of wheat straw for lactic acid fermentation were evaluated with glucose standards (Table 3.1). Results revealed that the acid pre-treated samples had the highest glucose concentration compared to the fungal pre-treated sample (Table 3.2)

Lactic Acid Production By Bacteria Fermentation Of Pretreated Wheat Straw

Lactic acid standards were prepared, and its absorbance was measured. The linear plot of the lactic acid standard (Figure 3.2) were used to determine the production of lactic acid from pre-treated wheat straw by bacteria. The prepared lactic acid standard as well as the its absorbance is represented in Table 3.3

Table 3. 2: Absorbance Of Lactic Acid Standards

S/N	Conc. of Lactic acid (mg/L)	Absorbance (490 nm)
1	0	0
2	10	0.018
3	20	0.026
4	40	0.031
5	60	0.051
6	80	0.071
7	100	0.112

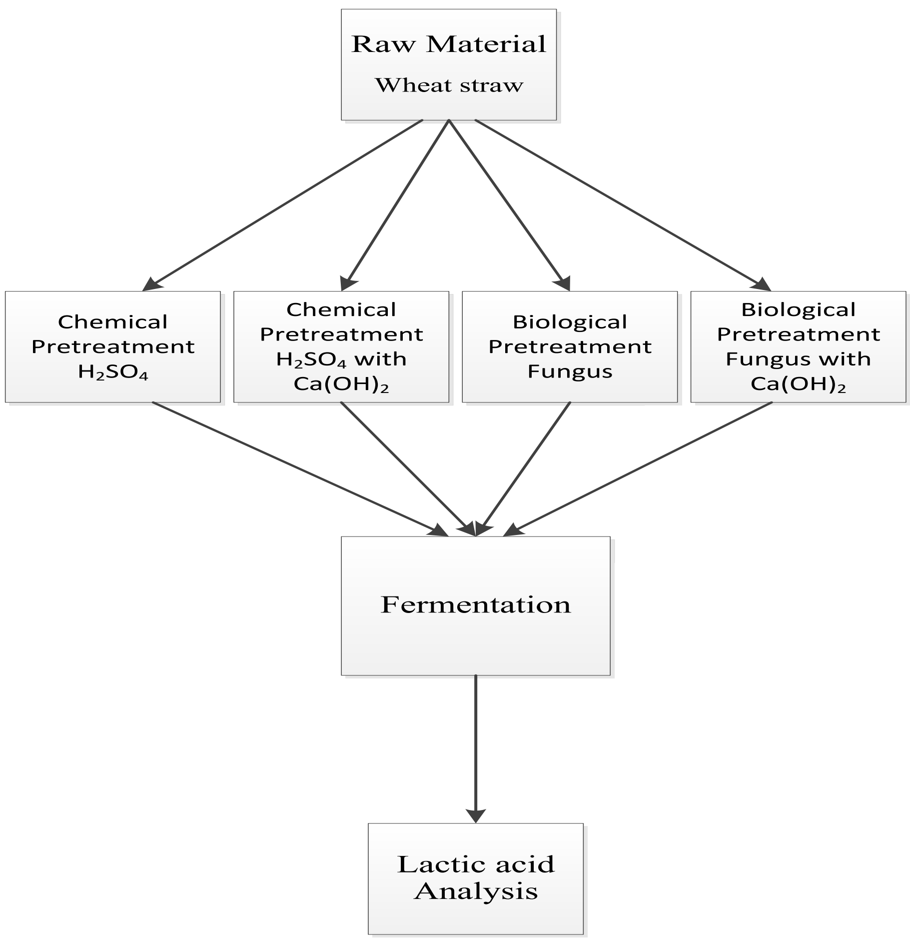

Figure3. 1: Process flow diagram

Effect Of Dilute Acid Pretreatment On Lactic Acid Production By Bacterial Isolates

Mixed bacterial culture fermentation of the acid pre-treated wheat straw revealed a diauxic yield of lactic acid over time. From the results presented in figure 3.4, it can be seen that lactic acid yield increased over time in an exponential phase until 48 hours and reached a steady state after 48 hour and after 72 hours there was a slight difference in productivity and finally reached to maximum concentration at 96 hours. The average concentration of lactic acid was significant, and a higher lactic acid yield was observed in fermentation using dilute acid pre-treated wheat straw.

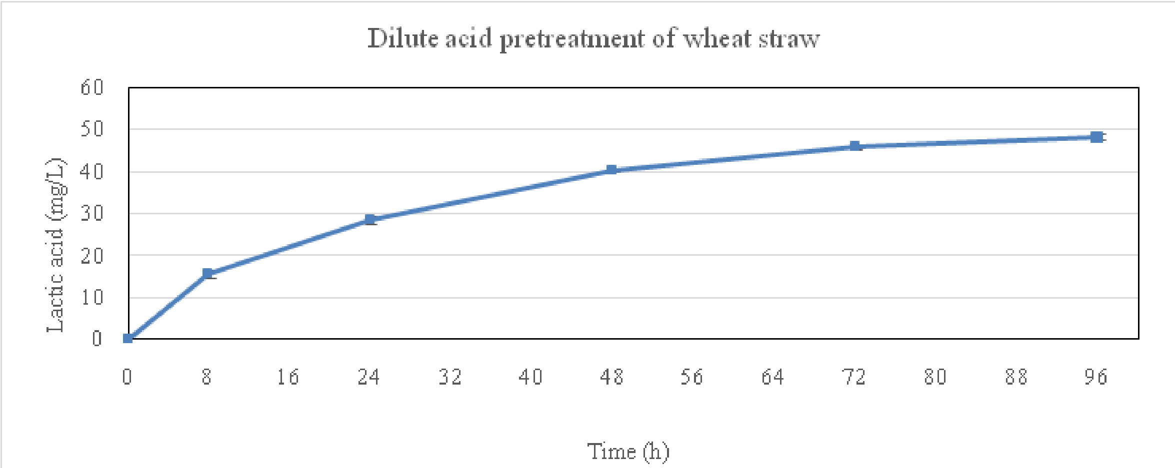

Figure3. 2: Lactic Acid Concentration Over Time Determined During Fermentation Containing Wheat Straw Pre-Treated With Dilute Acid.

Effect Of Fungi Pretreatment On Lactic Acid Production By BacterialIsolates

A graduallyaccumulating concentration of lactic acid was seen during the fermentation of bacterial strains that contained fungal pre-treated wheat straw. Though concentration increased consistently over time, there was no obvious steady state observed in fungal pre-treatment for lactic acid until 72 hours which started going into steady state after that until 96 hours. The average difference of production was seen between 24 to 96 hours. An overall lower lactic acid production was observed in fermentation using fungal pre-treated wheat straw hydrolysate condition as shown below in figure 3.5

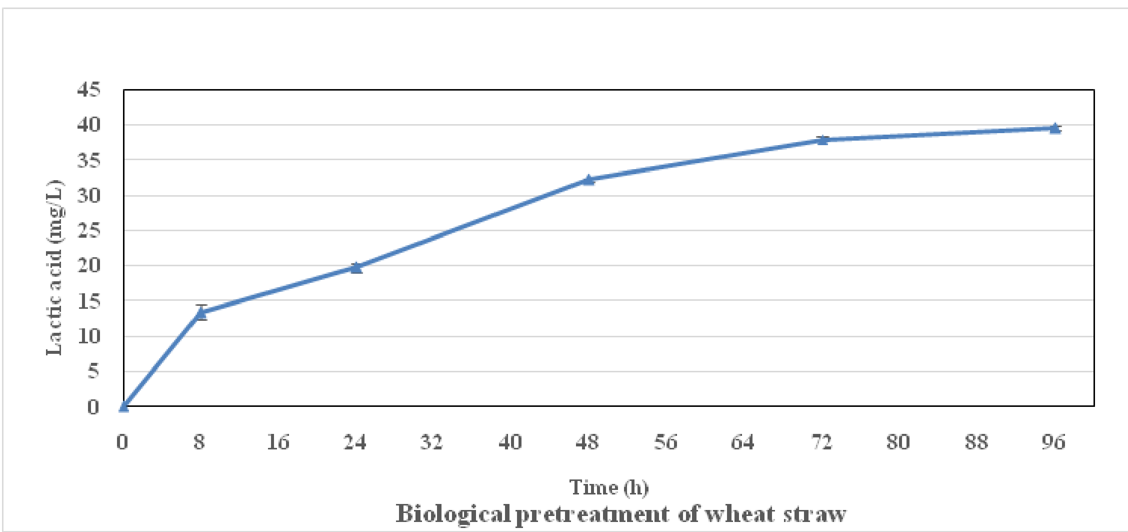

Figure3. 3: Lactic Acid Concentration Determined Over Time During Fermentation In Condition Containing Wheat Straw Pre-Treated With Fungi.

Effect of ca (oh) $_2$ On Dilute Acid pretreated wheat straw for lactic acid production by bacterialisolates

From Figure 3.6 calcium hydroxide was added with the aim of increasing concentration of lactic acid, in order to achieve the maximum production of desired product.Ca (OH) 2 has the ability in fermentation process to enhance the desired chemical production. The production of lactic acid increased slowly by the day and never reached a significant production rate. The concentration of lactic acid in different experiments with effect of Ca (OH) 2 on dilute pre-treated wheat straw was not enhanced dramatically. However, Calcium hydroxide addition in dilute acid pre-treated wheat straw adversely affect the production of lactic acid during fermentation and the efficiency of bacteria to produce lactic acid was significantly decreased in the presence of calcium hydroxide. An overall lower lactic acid yield was observed in fermentation withCa (OH) 2treatment of dilute acid pre-treated wheat straw as compared to only dilute acid pre-treated wheat straw.

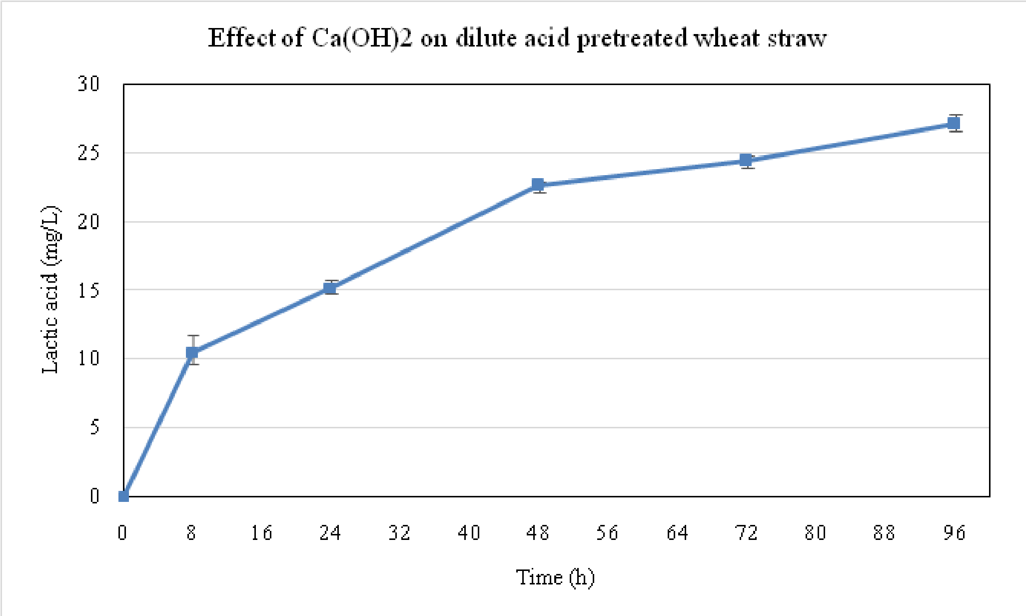

Figure3. 4: Effect of ca (oh) $_2$On Dilute Acid Pre-Treated Wheat Straw.

Effect of Ca (OH) $_2$ on Fungi Pretreated Wheat Straw For Lactic Acid Production By Bacterial Isolates

From Figure 3.7 The addition of calcium hydroxide in biological pre-treatment minimize the production of lactic acid. There was a decrease in lactic acid production due to lesser availability of nutrient (glucose) for the bacteria or as a result of the presence of Ca (OH) 2. Therefore, a subtle increase in lactic acid concentration can be seen until the final stage. The average value of concentration was lower, there was a steady state observed with the Ca (OH) 2 effect after 48 hours along with gradual increase until 96 hours. The lactic acid concentration was slightly higher in dilute acid pre-treated wheat straw with Ca (OH) 2 than inCa (OH) 2co-treatment onfungus pre-treated wheat straw. Overall, Calcium hydroxide addition in funguspre-treated wheat straw showed negativeeffects on the production of lactic acid in bacterial isolates and a reduced uptake of glucose by bacteria.

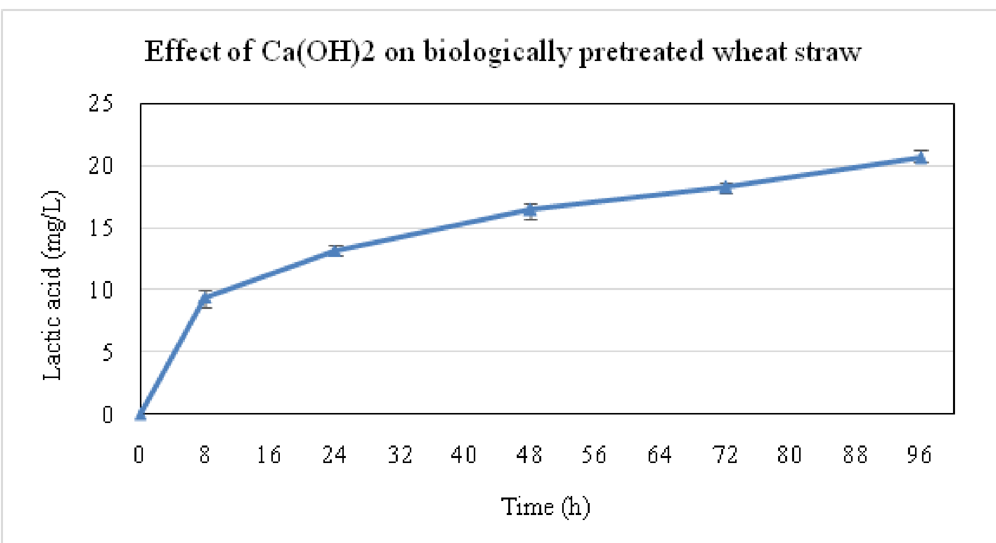

Figure3. 5: Effect Of Ca (Oh) $_2$ On Fungi Pre-Treated Wheat Straw.

Comparison for The Effect of The Different Substrate Pretreatment Methods and Effect ca (oh) 2 on Lactic Acid Production

Figure 3.8given below, shows that lactic acid yield was highest during fermentation of bacterial isolates with dilute acid pre-treated wheat straw compared to the biological pre-treatment method. Fungal pre-treated wheat straw condition produced lower lactic acid as compared to the acidpre-treated wheat straw. However,adverse results were seen in conditions containing an additional pre-treatment of calcium hydroxide. Lactic acid production decreased significantly in the presence of $Ca(OH)_2$ due to lesser availability of sugar from hydrolysate. Alkaline pre-treatment has shown to yield increased amount of sugars from wheat straw in older studies. However, results reported hereby proves adverse and negative effects of alkaline treatment of wheat straw along with acid pre-treatment which may result in neutralization and reduced efficiency of saccharification. Initial concentration of lactic acid was comparable between conditions of single pre-treatment and Calcium hydroxide co pre-treatment. Though an obvious difference can be seen after 24 hours of fermentation when concentration between conditions started to diverge more going towards the end. Fermentation using fungi pre-treated wheat straw produce higher lactic acid than fungi + $Ca(OH)_2$pre-treated wheat straw.However, Acid + $Ca(OH)_2$ pre-treated fermentation was slightly higher compared to Fungi +$Ca(OH)_2$ pre-treated condition. Though overall results of highest lactic acid yield pointed towards the acid pre-treated wheat straws without $Ca(OH)_2$ effect as the strongest condition used in fermentation of bacterial isolates.Processing of lignocellulose can produce inhibitor compounds like lignin derivatives such as, furan derivatives ethanol and phenolic compounds. These inhibitor compounds were accumulated by co-pretreatment with calcium hydroxide and decreased the conversion of sugars and thus the lower production of lactic acid in $Ca(OH)_2$ pretreated wheat straw (Zhang, 2009) [17].

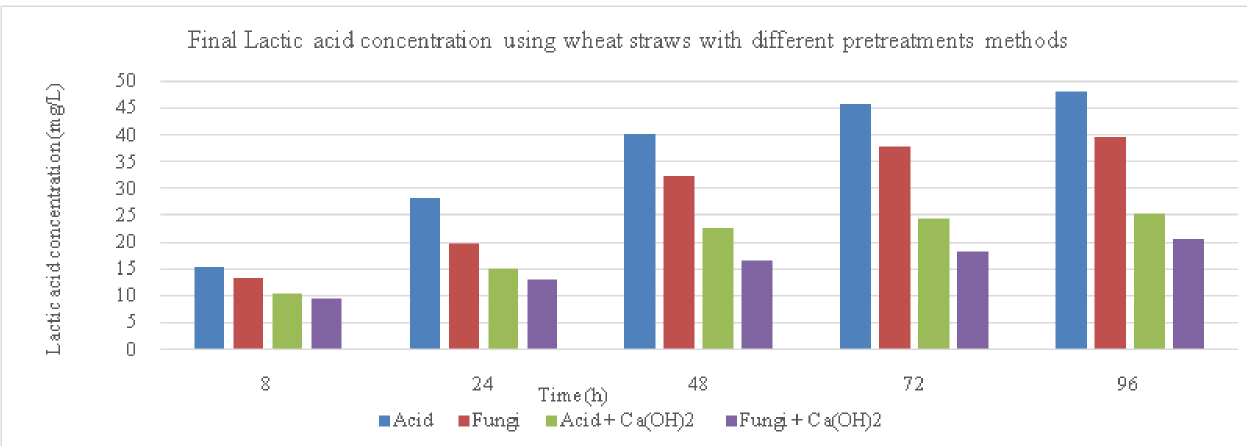

Figure3. 6: Comparison of Final Lactic Acid Concentration Using DifferentSubstrate Pre-Treatment Methods And Effect $Ca(Oh)_2$ On Lactic Acid Production

Comparison Of Glucose Concentration Yield Using Various Pre-Treatment Methods Of Saccharification

Glucose concentration was determined at different hours during fermentation in different pre-treated wheat straw hydrolysate conditions. A comparison of glucose concentration available in cell culture changing over time is presented in figure 3.9. Initial glucose concentrations obtained from various pre-treatment methods of wheat straw is presented at 0 hours. Dilute acid pre-treated wheat straw released the highest amount of glucose compared to rest of the methods while biological pre-treatment followed acid pre-treatment in glucose yield from wheat straw. Addition of Calcium hydroxide to dilute acid and fungus pre-treated wheat straw did not uplift glucose yield in wheat straw. On the contrary, calcium hydroxide addition reduced the efficiency of glucose consumption by cells and a higher residual glucose amount can be seen towards 96 hours in fermentation using calcium hydroxide co-treated wheat straw. Ineffectiveness of increase in glucose yield and reduced update by bacteria shows that calcium hydroxide co-treatment with dilute acid and biological pre-treatmentis not a productive way of co-treatment to release glucose from wheat straw. Consumption of glucose can be seen over time in all conditions, however, a higher residual glucose is left at 96 hours aligning with the lactic acid production in given fermentation conditions.

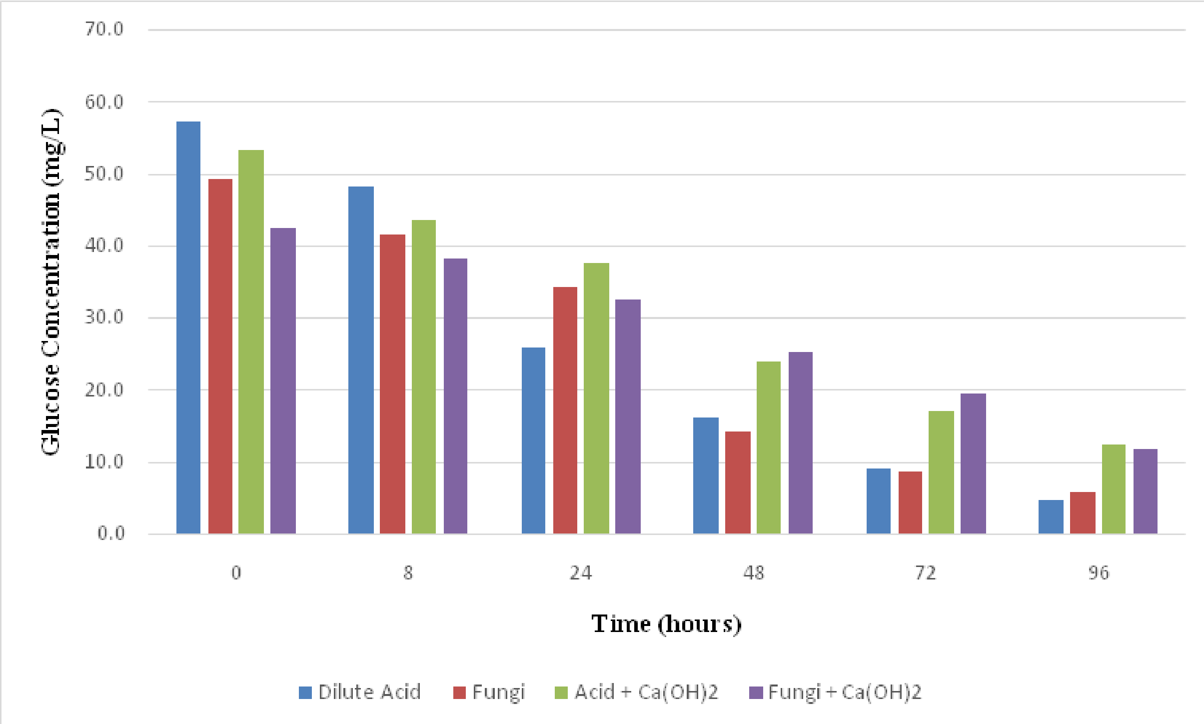

Figure3. 7: Comparison of glucose concentration over time in fermentation conditions with different pre-treatment methods

Comparison Of Concentration Of Lactic Acid Production At Ph 7, 8 And 9 By Bacterial Isolates During Fermentation

A comparison of lactic acid production is given below in figure 3.10. A linear production of lactic acid was seen at pH 7 for 96 hours of fermentation. Effect of pH was slight throughout fermentation at pH 7 with gradual increase in lactic acid production. However, fermentation at pH 7 produced significantly lower lactic acid in comparison to experimental conditions at pH 5 reported above. A lower initial concentration of lactic acid was seen at 8 hours which increased linearly until 48 hours of fermentation. An early steady state was observed after48 hours in lactic acid concentration, thus resulted in a lower concentration in the final hours. An overall production of lactic acid was lowered in fermentation at pH 8 as compared to experiments performed at optimal pH of 5 and 7 rendering pH 8 not suitable for producing efficient product from bacterial isolated during fermentation. In fermentation at pH 9 lowest lactic acid was seen since the start of fermentation at 8 hours which followed the same pattern of gradual linear production in next hours while a steady state was observed during fermentation at pH 9 at 48 hours. Final lactic acid concentration of lactic acid below was lowest at pH 9 fermentation as shown in figure An obvious difference of concentration can be seen between pH 7, pH 8 and pH 9. High pH effectsculture's viability and growth, thus its abilities to produce the product like lactic acid. Highest concentration of lactic acid was achieved at pH 5 which was used as an optimal pH to carry out the experiments reported above. The lowest lactic acid concentration was achieved at pH 9. Though, pH 8 and 9 were slightly comparable throughout towards the end of fermentation, however, it also shows its lethal effect to bacterial cell growth and metabolism.As fermentation was carried out at higher pH conditions, itadversely affected the efficiency of bacterial isolates to produce lactic acid.A high alkaline pH of 7, 8 and 9 is able to neutralize the lactic acid available, since the Actual pH of falls to 4-5 when lactic acid is produced, but when pH is raised, the alkaline effect of pH has inhibitory effect on the lactic acid production and start neutralizing the produced lactic acid (Hetényi, 2011).

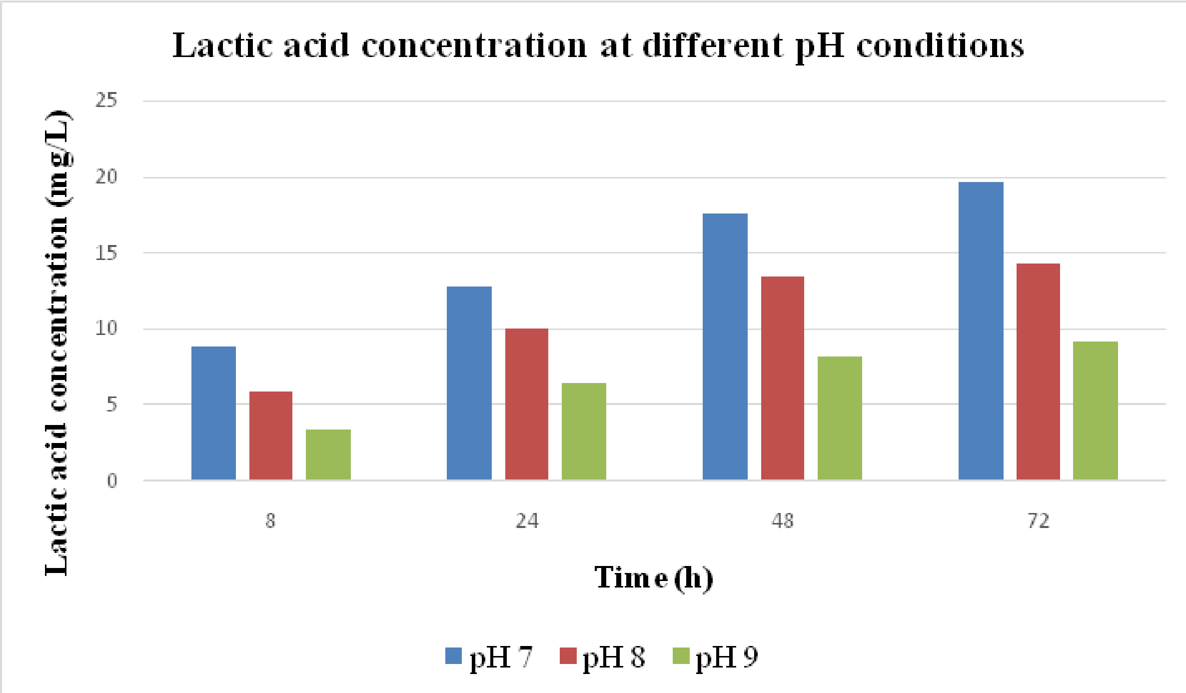

Figure3. 8: Comparison of lactic acid concentration produced in fermentation at pH 7, 8 and 9

Table 3. 1: Comparison Of Lactic Acid Concentration From Renewable Resources

Parameter					This Study
Substrate	Corn Stover	Cheese Whey powder	hardwood pulp	Corn Stover	Wheat Straw
Pretreatment method	Chemical	Biological	Chemical	Biological and chemical	Chemical, Biological
Glucose concentration	42 g/L	184.67 g/L	55.2 g/L	80g/L	57.2 mg/L, 49.2 mg/L
Lactic acid concentration	40 g/L	113.8 g/L	48.9 g/L	45 g/L	48,14 mg/L, 39.56 mg/L
References	Invalid source specified.	((Peng liu, 2018)	Invalid source specified.	Invalid source specified.	

Comparison Of Total Lactic Acid Yield In Different Fermentation Conditions

A comparison of final lactic acid concentration in all experimental conditions reported above can be seen in fig 3.11in order to evaluate the efficiency of different pretreatment methods applied on wheat straw and the effect of various pH conditions to produce the maximum concentration of lactic acid from bacterial isolates during fermentation. Fermentation from wheat straw with dilute acid pretreatment method of saccharification produced the highest concentration of lactic acidand consumed the highest amount of glucose. This is because dilute acid has the potential to release more sugar from wheat straw.Addition of calcium hydroxide did not enhance the productivity as compared to conditions without calcium hydroxide treatment, but it also had an adverse effect in the production of lactic acid by decreasing the efficiency of bacteria to produce the product. Use of wheat straw with calcium hydroxideco-treatment along with acidic and biological pretreatment in fermentation reduced the yield of desired product.

Highest concentration of lactic acid was achieved at an optimal pH of 5 which was used to carry out fermentation with different pretreatment methods. However, much lower lactic acid yield was obtained with fermentation at pH 7, 8 and 9, where pH 9 at final 72 hours, resulted in lowest production of desired product after coming to steady state in last hours.

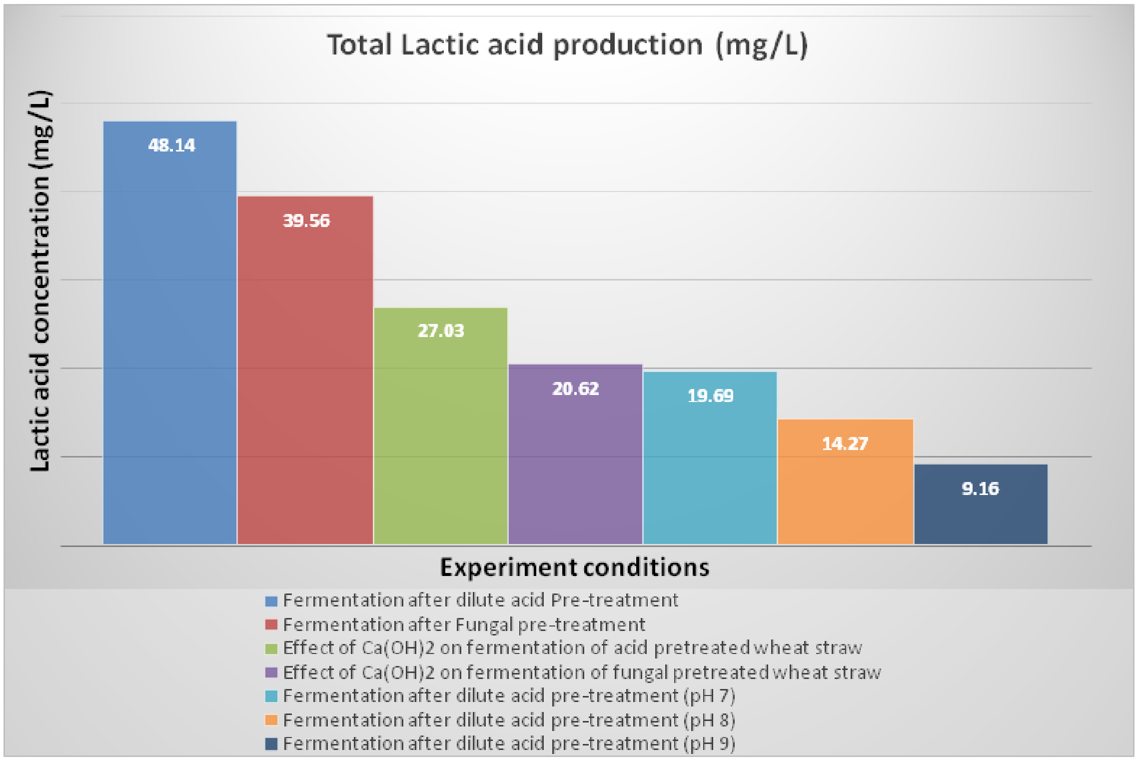

Figure 3.9: Comparison of final lactic acidyieldin different fermentation conditions

Table 3. 2: Lactic acid yields obtained from fermentation using pretreated wheat straws

	Dilute Acid	Fungi	Acid + $Ca(OH)_2$	Fungi + $Ca(OH)_2$
Product yield, Yp/s (mg/mg)	0.915	0.908	0.66	0.67
Percent Yield (%)	84.20	80.40	50.84	48.53

Lactic Acid Yield

As shown in table 3.5 above, Product yield from substrate and percent yield has been calculated for lactic acid produced during fermentation. Product yield for dilute acid pretreated fermentation was highest with a value of 0.915 mg/mgand the percent yield of 84.2%, while fungus pretreatment of wheat straw provided the product yield of 0.908 and percent yield of 80.40%. However,-co-pretreatment of acid with calcium hydroxide and fungi with calcium hydroxide provided lowest product yield of 0.66mg/mg and 0.67 mg/mg respectively and the lowest percent yield of 50.84% and 48.53%.

Conclusion

Chemical and biological pretreatment of wheat straw was carried out to convert biomass into sugars for use in fermentation process of lactic acid production. Different fermentation conditions were performed from differently pretreated wheat straw hydrolysate while the sugar and lactic acid analysis was performed throughout the process with intervals.All the fermentation processes were carried out at an optimum pH of 5 during this experiment after analyzing lactic acid production at pH 5, 7, 8, and 9.Increased pH during lactic acidfermentation has an inverse effect on cell culture and yields lower lactic acid concentrations.Moreover, the effect of calcium hydroxide co-pretreatment along with dilute acid and fungal pretreatment on the yield of sugar and lactic acid was evaluated. Total initial sugar yield from dilute acid pretreatment was 57.2 mg/L while the fungal pretreatment yield was 49.2mg/L. Co-pretreatment of dilute acid and fungal pretreated wheat straw with calcium hydroxide released a comparable amount of glucose. No significant difference of initial glucose concentration was found after the co-pretreatment with calcium hydroxide. However, a decreased consumption rate of glucose by bacteria was observed during fermentation using calcium hydroxide co-retreatment on both dilute acid and fungal treated wheat straw. Dilute acid pretreated wheat straw provided the highest yield of lactic acid of 48.14 mg/L as compared to the fungus pretreated wheat straw yield of 39.56 mg/L. Use of calcium hydroxide with chemical and biological pretreated wheat straw hydrolysate lowered the total lactic acid production significantly as compared to individual pretreatments without calcium hydroxide.

It was found that saccharification of wheat straw using chemical pretreatment method provides enhanced amount of converted glucose whereas conversion of biomass in biological pretreatment followed the chemical pretreatment. Whereas, adverse effects were seen on lactic acid production in fermentation using calcium hydroxide co-pretreated wheat straw hydrolysate.

Demand for lactic acid has been increasing rapidly in various industries recently. There have been many ways of lactic acid production. This methodology and technology sustain many process in chemical industries like the production of many organic acid like lactic acid and others, global use of this technique can produce bio-energy to fulfil the requirement of low cost energy.With the increasing demand of environment friendly production using fermentation process, it is necessary to move towards the use of inexpensive and cost-effective raw materials. The major barriers towards the production of lactic acid in industry are the larger amount of production and high cost of production. To explore the best and cheapest resources, this study evaluated saccharification methods for lignocellulose conversion into pentose and hexose sugars. Findings of this study can be utilized for an environment friendly production of lactic acid using the cheapest resource of wheat straw which is easily and abundantly available in low costcan be used after pretreatments with dilute acid.However, study was performed on a small scale in shake flask and presented limited data, though future studies can be performed on bioreactor scale for confirmation with larger data. Furthermore, efforts for developing the procedure to produce higher concentrations of lactic acid for industrial production would be highly useful. More efforts for continuous process development should be made to further develop utilization approaches of the method. The results presented in this study suggest the importance of evaluating commercial potential of using pretreated wheat straw as substrate in the large scale production of lactic acid [19-64].

Declaration

Ethical Approval And Consent To Participate

The research was approved by the ethics committee of Cyprus international university. There was no coercion whatsoever, participation did not have any influence on the care provided or received and there were no financial incentives for the investigators nor for the patients included.

Consent For Publication

Not applicable

Availability Of Data And Materials

All the materials were used in the microbiology laboratory of Cyprus international university.

Competing Interests

Cyprus international university paid for this research.

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