Production Of Lactic Acid From Tamarind Kernel By Lactobacillus Casei.

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ABSTRACT: In present work lactic acid is produced by microbial fermentation of sugar obtained from tamarind kernel powder (TKP) and its feasibility has been studied for use as raw replacement for glucose. Saccharification of tamarind seed powder is carried out by acid hydrolysis using sulfuric acid (H_2SO_4). Optimization of acid catalyst concentration and hydrolysis time was carried out in a steam autoclave to get higher yield in short time. The highest yield of reducible sugar of 0.71 g/g of substrate has been obtained by using 0.5 N H_2SO_4 at 121°C, in 30 min. This tamarind kernel hydrolyzate (TKH) after neutralization and charcoal treatment is used as a source of reducible sugar for production of lactic acid. Four commonly used lactobacillus strains were used to study the suitable microorganism for the fermentative production of lactic acid from TKH sugar media. Out of them the strain giving better result Lactobacillus casei -2125, is selected for the further studies. The rate of glucose consumption and lactic acid production by Lactobacillus casei -2125 for standard glucose media and for TKH sugar media was studied in batch fermentation. The results for the standard media of glucose and the TKH was comparable and show nearly 90% conversions in 66 to 72 hour of reaction time, respectively. It gives production of lactic acid up to 81 gl^{-1} with productivity rate of 1.12g / l / hr. A yield of 0.58 g of lactic acid is obtained from each gram of TKP. It shows that the TKP can be used as a suitable and cheap raw material for the lactic acid production.

KEY WORDS: Acid hydrolysis, Agro waste biomass , Fermentative production of Lactic acid, L. casei, Tamarind kernel powder.

1. INTRODUCTION

Lactic acid (LA), also known as milk acid, is the most abundantly occurring organic acid in nature. It was first isolated in 1780 by a Swedish chemist, Carl Wilhelm Scheele, but actually first produced commercially in Littleton, Massachusetts, USA by Charles E. Avery in 1881. Lactic acid is a versatile chemical having numerous applications in pharmaceuticals, cosmetics, food industry [1],[2]. It is also used as raw material for the production of the chemicals like, lactate ester, propylene glycol, 2-3 pentane dione, propionic acid, acrylic acid, acetaldehyde and lactide. These chemicals find various applications in food, pharmaceutical, polymer, textile industry [3],[4]. In recent decade it finds wide application, as monomer for biodegradable polymer, polylactic acid [5]. The lactic acid market has been estimated to be 3.3 million tons by 2015, showing that the demand is continuously increasing, with a growth rate of 8% per annum. Its demand is continuously growing in the market. But its use for polymer field applications is restricted by the cost of pure L-lactic acid [6]. Lactic acid has been produced from a variety of carbohydrates, including starchy and lignocellulosic biomasses, depending on the substrate availability in the producing country [1]. Cost of raw material, pretreatment and saccharification of raw materials by physicochemical and enzymatic treatment are one of the bottleneck processes for cost-effective lactic acid production [7]. To minimize its cost of process and to give new cheap alternative raw material to the conventional starch or sugar utilization in production of lactic acid, we used - acid hydrolyzed tamarind kernel powder (TKP) for lactic acid production. Tamarindus indica L., commonly known as tamarind tree is one of the most important multipurpose tree species in the Indian sub-continent [8]. The tamarind fruit pulp has been an important culinary ingredient in India for a very long time. Almost all parts of the tree find some use, in food, chemical, pharmaceutical and textile industries, and as fodder, timber and fuel. In India, tamarind (Tamarindus indica L.) is a very beneficial tree, growing abundantly in the dry tracts of central and south Indian states [9]. Indian production of tamarind pulp is about 3 lakh (0.3 million) tones per year [10]. The hard pod shell is removed (deshelled) when the fruit is ripe and the fruit is the major acidulant used in the food preparations. Out of the fruit pulp, its seed has the 25-40 % of total dry weight [11]. So about 75-120 thousand tones of the tamarind kernel seed is produced annually. It finds applications in textile, pharmaceuticals and in packaging application. It has nearly 70% of starch content. Tamarind seed polysaccharides, also called glycans, consist of mono saccharides and their derivatives. It contains galactose, xylose, and glucose in the proportion of 1: 2.5:2.8 respectively and about 2-3 % of the polyfuranose [12]. In this work, the ability of lactic acid producing bacteria (LAB), to produce lactic acid from tamarind seed powder (TKP) was investigated. Sulfuric acid is used as the inorganic acid catalyst as the productivity of sugar is equivalent to similar conc. of hydrochloric acid and higher than the phosphoric acid [13]. TKP was pretreated to remove and hydrolyzed by various concentrations of inorganic acid (H_2SO_4) to get the optimum concentration of acid giving highest sugar content in the hydrolyzate. The effects of hydrolysis conditions, including acid concentration, temperature and treatment time, on sugar yield from tamarind kernel powder were studied. Four lactobacillus strains were used for checking the suitable microorganism for the fermentative production of lactic acid from TKH sugar media. Out of the four strains used, the strain showing best performance with respect to the utilization of sugar and lactic acid production has been selected for the further study. Its fermentative capacity to produce lactic acid from standard glucose media and TKH media has been compared with respect to the rate of sugar consumption, microbial cell growth and their effect on lactic acid production rate is studied and the results have been presented in this paper.

2. MATERIALS AND METHODS

2.1 Chemicals

Tamarind kernel powder (TKP) procured from local markets, of Mumbai, India (during season of 2013 March). L-lactic acid 99.9%, sodium di-hydrogen phosphate di-hydrate, phosphoric acid, yeast extract, peptone, deMan, Rogosa and Sharpe (MRS) broth, MRS agar, glycerin, D-glucose, K_2HPO_4, sodium acetate, diamonium citrate, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, Tween-80 (poly sorbit-80) and other nutrients were purchased from HiMedia, India. All the chemicals used were of analytical reagent (A.R.) grade and used as received.

2.2 Microorganism

L. bulgaricus-2056, L. delbrueckii-2025, L. casei -2125, L. casei var.rhamnosus -2364 was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, CSIR-India. The strain were incubated for 24 hour and stored in MRS broth with 70% glycerin at -80^{0}C.

3. EXPERIMENTAL

3.1. Analytical Methods

3.1.1. Raw Material Analysis

Total sugar content was determined by a modified phenol sulfuric acid method [14]. A 0.5 ml sample of ethanol extracted TKP was mixed with 0.1 ml of 5% phenol reagent and 2.5 ml of concentrated sulfuric acid. After 30 min of incubation at room temperature (25°C), the absorbance at 490 nm was read with a UV-Vis spectrophotometer, Chemito, Spectrascan UV2700, Double beam and compared with a standard curve established by glucose. Total reducible sugar was determined by 3,5 di nitro salicylic acid method (DNSA) [15]. Absorbance of the sample was read at 540 nm by double beam UV-Vis spectrophotometer. Total phenolic compounds present in the raw material were determined by using foline-ciocalteu reagent (FCR). In 2 ml of filtrate solution, 10 ml of FC reagent (diluted 1:10 ratio) was added followed by addition of 8 ml of 75 g/l Na_2CO_3. Then total volume of reaction mixture was made up to 20 ml with distilled water. Reaction mixture was kept at room temperature for 2 hrs and then measured the optical density at 765 nm. Vanillin was taken as standard to estimate total phenolic compounds in biomass [16]. Crude fiber of the sample were determined by the Proskeys method [17]. Other analysis of raw material for determination of moisture content, fiber content, lipid content, ash content, nitrogen content was done according to the standard methods of analysis of AOACS [18].

3.1.2. Cell Count

Cell growth during fermentation was determined aseptically sampling an aliquot of the cultures. The cell growth was measured by taking optical density of the sample media at various time intervals by spectrophotometer. To measure cell growth, a UV-Vis spectrophotometer, Chemito, Spectrascan UV2700, Double beam was used with its detection wavelength set at 620 nm. Samples were diluted ten times with 0.1M HCl to dissolve the $CaCO_3$ particles. The optical density values are corrected with the viable cell count by haemocytometer.

3.1.3 Detection Of Lactic Acid

High-performance liquid chromatography (HPLC) was employed to analyze lactic acid present in the fermentation broth, using HiQsil, C-18 HS reverse phase ODS column (KYA TECH, HPLC Column, Japan 240 mm x4mm, 0.4 µm particle size) maintained at 40°C by a column heater. The detector used is Waters 2489 (UV/Vis) Detector and 515 isocratic pump (Waters, Massachusetts, United States). The mobile phase was NaH_2PO_4 + H_3PO_4 10mM buffer system (pH 2.5) at flow rate of 0.8 ml min^{-1}. (19) Samples for HPLC were prepared by centrifuging samples at 5000 rpm for 10 min in an REMI bench top centrifuge R-4C DX (Remi, India), and the resulting supernatant was filtered through a 0.2-µm polyether sulfone (PES) membrane. For all the analysis 20 µl of diluted sample is injected. Concentrations of lactic acid were determined by comparison of area of sample with a standard curve generated using L (+) lactic acid (99%) stalk solutions.

3.2 Pretreatment Of Raw Material

The tamarind kernel powder (TKP) purchased from local markets, contains the excess of moisture and lipids (oil/fats). To remove moisture TKP was dried at 60 ^{0}C for the 18 hours and then it was sieved through a 200 mesh screen. It was extracted with petroleum ether (60-70^{0}C Fraction) in soxhlet to prepare defatted TKP. It takes six to eight cycles to get the highest extraction of lipids. The extracted residue is dried and powder was stored in an airtight container at room temperature until use.

3.3. Hydrolysis Optimization Of TKP

Carbohydrates present in a TKP, in the form of starch, celluloid's, hemicelluloses, fibers is not suitable source for the bacterial diet. So it was converted in to the soluble sugars by hydrolyzing with mineral acid (sulfuric acid). Hydrolysis of the pretreated TKP is done in autoclave at 120 ^{0}C temperature and 15 psi pressure for 80 min. Hydrolysis of TKP was performed by adding 20% w/v of TKP in sulfuric acid solution. Different concentration of acid solution ranging from of 0.1 N, 0.25 N, 0.5 N, 0.75 N, 1.0N and 2N is used for the hydrolysis. Aliquots from the reaction media were taken at defined time intervals and analyzed by DNSA method for the reducible sugar content, using the same procedure as described above. After the completion of reaction, hydrolyzed liquid was filtered to separate residue of TKP. Filtrate was neutralized with calcium carbonate ($CaCO_3$). It was heated at 70 ^{0}C for 30 minute and cooled to get complete precipitation of the $CaSO_4$. It is centrifuged at 5000 rpm for 10 minutes and supernatant liquid is separated. It is treated with 1% w/v activated charcoal to remove any inhibiting substance and colored substances.

3.4 Fermentation

3.4.1. Selection Of Lab Strain

All the four LAB strains were grown on MRS Broth (HiMedia), containing 21.0 g of media per liter of MilliQ water, filtered through a sterile 0.22 µm PES membrane. After inoculating it was incubated at 38 ^{0}C for 24 hr and

stored in the 2ml Eppendorf tube in a 70% glycerol at -80 ^{0}C until use. Growth media containing (g l^{-1}) dextrose 10g; protease peptone 2g; yeast extract 2g; tween-80 0.1g; ammonium citrate 0.02g; sodium acetate 0.01g, $MgSO_4$ 0.01g; $MnSO_4$ 0.05g and K_2HPO_4 0.5g;. Media was sterilized by autoclaving at 121 °C and 15 psi pressure for 15 minute. It is inoculated with loop of cell in 20 ml of medium and incubated at 38°C for 24 h with shaking at 150 rpm. For the TKH culture, source of sugar is the 10 g l^{-1} of reducible sugar from TKH. Shake flask experiment were carried for the selection of the LAB strain without pH control, in media containing 10 g l^{-1} of TKH sugar. It is inoculated with 5 v/v of viable cell in 100 ml of medium in 250 ml flask and incubated at 38°C for 48 hour with shaking at 150 rpm. Samples were collected at zero time and after the completion of 48 hours.

3.4.2. Batch Fermentation

Batch fermentations with two different carbon sources, glucose as the typical carbon source and tamarind kernel hydrolyzate (TKH) were studied. The production media contains 100 g l^{-1} of reducible sugar. All batch fermentations were carried out in 1 l glass reactor containing 500 ml fermentation media. The temperature was maintained by water circulation through the external jacket, maintained at 38 °C. Nitrogen was purged continuously to maintain the micro aerobic condition. The pH was monitored by digital pH meter and it is maintained at ~6.0 by adding sterile $CaCO_3$, which is kept at 50% w/w of initial sugar concentration. The stirring was maintained at 150 rpm. Fermentation growth and production kinetics was studied by taking sample between various time intervals. Each sampling involved a 3 ml extraction of culture liquid with a sterile pipette. An aliquot of each sample was divided into two parts. One part was acidified to pH 3 with 1N sulfuric acid to precipitate calcium sulfate and to liberate lactic acid from calcium lactate. It was centrifuged at 5000 rpm for 10 min. The supernatant liquid after filtration through 0.2 µm PES membrane was used for sugar analysis and for lactic acid detection. The second portion is acidified by hydrochloric acid to dissolve unreacted calcium carbonate and precipitated calcium lactate. It is diluted ten times and used for cell count determination by spectrophotometer, by measuring its optical density (OD) at 620nm. All experiments were carried out in triplicate and data were subjected to a one way analysis of variance (ANOVA). Values expressed were ± standard deviation (SD) for numerical values in table and graphs with error bars.

4. RESULTS AND DISCUTION

4.1. Pretreatment Of Raw Material:

The pretreatment method of de-fatting and drying, enhances the active content of raw material and removes the less valuable components. De fating by the soxhlet extraction separates the crude oil/ fat from the TKP which has several applications in paint, varnishes and burning oil [20]. So we get the byproduct of TKP which can help to reduce its cost. Drying of the material enhances its self life and helps to store the material for long duration. These processes make the sieving through 200 mesh size more easily by preparing free flowing material. These pretreatment processes are beneficial for the hydrolysis by acid catalyst, by giving more porous and fine material.

TABLE 1.
Proximate raw material composition chart

Constituent	Raw Tamarind Kernel Powder [a] in Percentage	Dried and Defatted Tamarind Kernel Powder [a] in Percentage
Moisture	7.6 ± 0.22	0.55 ± 0.11
Protein	12.4 ± 0.27	15.3 ± 0.08
Fat/oil	5.4 ± 0.15	0.3 ± 0.02
Crude fiber	4.7 ± 0.12	4.8 ± 0.11
Total Ash	1.6 ± 0.08	1.9 ± 0.08
Total sugar	72.1 ± 1.20	76.0 ± 1.44
Reducing sugars	68.0 ± 1.15	72.5 ± 0.8
Phenolic content	0.9 ± 0.15	0.09 ± 0.04

a. *Mean values of triplicate analysis with standard deviation*

4.2. Hydrolysis Optimization Of TKP

Hydrolysis using the acid catalyst at is beneficial over the enzyme catalyst as it gives higher yield of product in relatively short time [13]. Also the cost of the mineral acid and its easy availability make it good candidate for the industrial processes. Hydrolysis using an acid at elevated pressure and temperature is beneficial over the other methods of hydrolysis, as it gives the higher yield in relatively short duration [21]. In present work temperature and pressure of 120 ^{0}C and 15 psi respectively was used for all the hydrolysis experiments. This makes acid activity higher at relatively small concentration. The lowest acid concentration that yielded the highest theoretical reducible sugar conversion of more than 95% of actual amount is 0.25N for 20% w/v in 40 minute (figure 1.). However, the use of a higher concentration of acid of 0.5N resulted in a faster conversion of TKP to sugar with the highest concentration of 98% conversion, completing hydrolysis within 30 minutes. Because the maximum hydrolysis yields of 100 g TKP was 72 g of reducible sugar, treatments with 0.5N H_2SO_4 generated maximum sugar concentrations (71 g) close to the maximum theoretical yield by 30 minutes. It is possible that the lowest acid concentration (0.25N) could still achieve the similar level of hydrolysis with longer treatment times (50 min); however, the production of by-products such as acetic acid, formaldehyde, furan is higher for longer reaction time. Although 1N and 2N acid produced the highest concentration of glucose in the shortest interval of time (20 min). But higher concentration of acid required higher amount of neutralizing agent and also gives more degradation of sugar and generates by-product waste[21]. Use of the H_2SO_4 is beneficial over the other acid as neutralization by the $CaCO_3$ gives the relatively insoluble salt of calcium sulfate ($CaSO_4$) and unreacted $CaCO_3$ precipitate. It makes the downstream separation process easy. Hence for the further experiment, acid concentration and time to fully hydrolyze the TKP was kept at 0.5N and 30 min respectively, for 20% w/v of TKP at 121^{0}C, at 15 psi pressure. This optimized condition was deemed acceptable as a pre-treatment of all TKP samples prior to fermentation.

After hydrolysis, acidic solution was neutralized by calcium carbonate and treated with 1% activated charcoal, to remove any colored impurities.

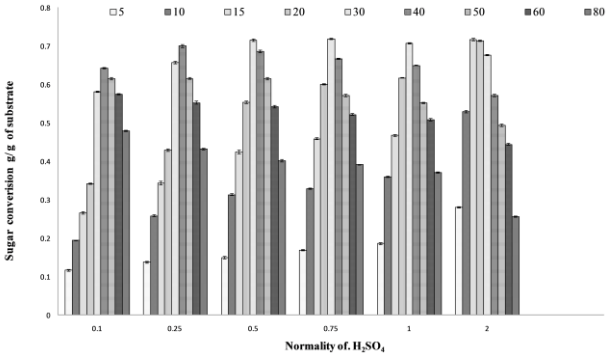


Fig.1. *Hydrolysis optimization of TKP at different sulfuric acid concentration.*

4.3 Fermentation

The four lactic acid producing bacteria (LAB) strains used in this study were selected as they produce a single isomer of lactic acid, they are commonly used in fermentation of agro wastes and they were most tolerant of the micro aerobic environment of the shake flasks [22]. Table no. 2 represents the observations of the fermentation at pH uncontrolled environment. It showed that variable growth for the all four strains with different yield of lactic acid. The two strains namely, L. bulgaricus-2056 and L. delbrueckii-2025 which are homo fermentative bacteria showed the lower consumption of sugar and also the less productivity of the lactic acid. While the L. casei -2125, L. casei var.rhamnosus -2364 which are the facultative bacteria showed the better utilization of the TKH sugar, with higher yield of lactic acid. These results are consistent to the behavior of these bacteria, as they show better tolerance for lower pH. Out of four strains L. casei -2125 is found superior with respect to sugar utilization and lactic acid production. So it is selected for the further studies in batch reactor.

TABLE 2
Fermentative capacity of lactobacillus species

Parameters	L. bulgaricus-2056	L.delbrueckii-2025	L. casei -2125	L. casei var.rhamnosus -2364
Process Time (Hr)	48	48	48	48
Max. O.D.$_{620nm}$	2.49	2.67	2.92	2.79
Maximum Lactic acid conc. (g l^{-1})	2.3	2.5	3.8	3.4
Maximum yield of lactic acid to sugar (%)	23	25	38	34
Final pH	4.4	4.3	3.6	3.8

4.4. Batch Fermentation

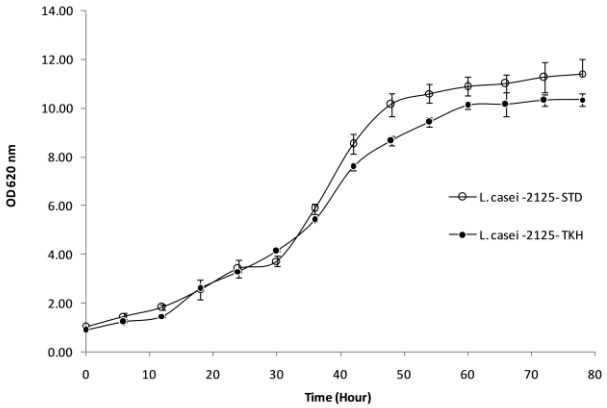
4.4.1. Effect Of Neutralizing Agent

Several studies have confirmed the beneficial effects of pH control during production of lactic acid [23], [24]. There are different methods to control pH, but the simplest to employ in a small-scale experiment without detrimental effects to the product is to neutralize the hydrogen ions as the pH drops below a set value. Another group investigated the effects of $Ca(OH)_2$, NH_4OH and NaOH as neutralizing agents for efficient recovery of lactic acid, and demonstrated that divalent cations (Ca^{2+}) was more effective in neutralizing the cultures relative to monovalent (Na^+ and NH_3^+). Therefore, choices of appropriate neutralizing agent are crucial for cost effective and efficient production of organic acid with high concentration and high yield as well as high productivity. As regards lactic acid fermentation process, either $Ca(OH)_2$ or $CaCO_3$ appears generally used on industrial scale, which makes downstream processes of recovery and purification easier. Also they are cheaper than other neutralizing agents (e.g., NH_4OH or NaOH) [25] With a working pH range of 4.5–6.5, the pH set points designated for this experiment were nearly pH 6.0. As Lactobacillus strains are generally tolerant to high salt environments [1], the addition of base could increase the medium osmolarity causing the bacterial cells to swell and burst, so the addition of $CaCO_3$ was beneficial as it is insoluble in neutral pH and produce weakly soluble calcium lactate, which helps to keep lower concentrations of salts maintaining the pH throughout the experiment without damaging the cells [26]. L. casei -2125 is a facultative hetero fermentative species that performs both fermentations. It consumes hexose sugar by homo lactic pathway while pentose is consumed by hetero lactic pathway [27].

4.4.2. Growth Of Bacteria

The rate of growth of bacteria in both the media was comparable with slightly lower rate of growth for the TKH sugar culture media. It shows the effect===ive utilization of sugars by bacteria and also there is no inhibitory effect of the TKH sugar media. Maximum cell growth is observed at 50 hour duration after which there was a stationary phase, giving negligible growth.

FIGURE 2
Cell growth in standard glucose media and TKH sugar media



4.4.3. SUGAR CONSUMPTION

Studies showed that during the fermentation of lactic acid, the presence of glucose affected the consumption of xylose, which was recognized as the repression of xylose uptake by glucose [28]. Hence, to evaluate the effect of mixture of sugar utilization by L. casei -2125, fermentations were conducted in the medium with glucose as sole carbon source, and compared its results with the TKH sugar. For the carbon source of hexose sugar, the single pathway of sugar utilization is preferred for both the culture media. As TKH contains nearly 60% of the hexose sugar (glucose and galactose) it can be entirely feasible for L. casei -2125 to consume the TKP sugar and the glucose with the similar rate within 40 hour of fermentation for both the cultures. The average rate of substrate consumption in media containing glucose (1.34g l^{-1} h^{-1}) was higher than that in TKH sugar media (1.25 g l^{-1} h^{-1}). While consumption of the rest of sugar in glucose media takes place in 60 hours, TKH sugar media shows still unconsumed sugar of nearly 10% of initial value. It comes to nearly saturation point at this level and the rate of consumption becomes very low. It is because as compared to glucose, the conversion of xylose requires additional enzymatic steps. When cells are exposed to xylose there is a lag of time before the enzymes required for ingestion appear, as some enzymes are inducible [29]. In the presence of glucose, genes required for xylose utilization are repressed. The several studies have shown that most microorganisms could not metabolize xylose and glucose simultaneously. Xylose began to be consumed when there was no glucose in the medium [30], [31]

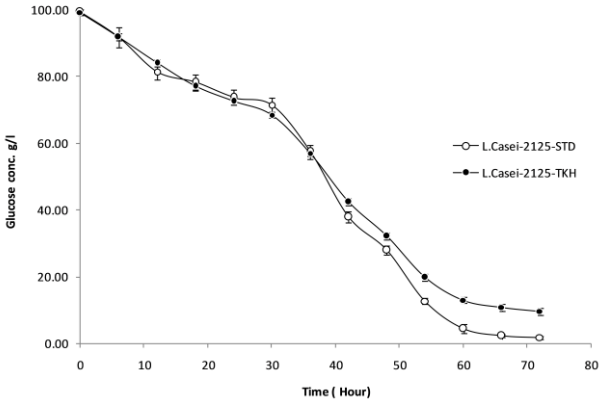


Fig. 3.*Consumption of sugar in standard glucose media and TKH sugar media*

4.4.4. Lactic Acid Production

The higher lactic acid production for a hetro fermentative species is unusual, but it is common for facultive hetrofermentator species and it always generate minimal amounts of acetic acid and formic acid. In fact, hetro fermenters utilize hexoses via the Embden–Meyerhof pathway [32]. While catabolism of pentoses requires additional conversion steps through which they are transformed into metabolic intermediates of the pentose phosphate pathway. By this way, as an instance, xylose is transformed into xylulose and then phosphorylated to xylulose 5-phosphate [33] The glucose media showed almost 97 g l^{-1} of lactic acid production in less than the 72 hour and it gives the productivity of 1.34 g l^{-1} h^{-1}. While TKH sugar media gives the production of 76.8 g l^{-1} in the reaction time of 72 hour and it gives the productivity of 1.1 g l^{-1} h^{-1} .This is due to the slower rate of consumption of xylose sugar by the L. casei -2125. Thus almost 0.55g of lactic acid can be produced from each gram of tamarind kernel powder.

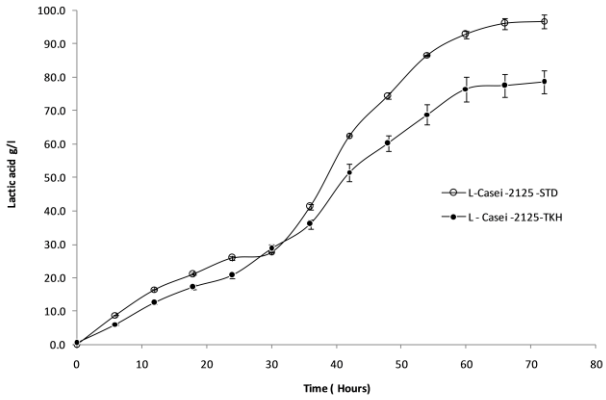


Fig. 4. *Production of lactic acid in standard glucose media and TKH sugar media*

5. CONCLUSION

In conclusion, hydrolysis of pretreated TKP by using 0.5N concentration of sulfuric acid at 120^0C temperature and 15 psi pressure gives nearly 98 % of conversion. It gives yield of 0.71g/g of reducible sugar per gram of substrate. A simple hydrolysis process involving treatment of 20 % w/v of TKP with 0.5N H_2SO_4 followed by neutralization with $CaCO_3$ is economical process. Fermentation of TKH using L.casei-2125 at pH control environment produced close to 79 g of lactic acid in 72 hours with the rate of production of ~ 1.12g / h / l. It reflects the yield of 0.58 g of lactic acid per gram of TKP (~ 0.58g g l^{-1}). The lactic acid to glucose ratio (LA/total sugar = 0.87) observed in this work was lower than yields observed with L. casei-2125 using glucose media in similar time. But as compared to the cost of the glucose or starch it is really economical. Thus the tamarind kernel powder can be used as cheap alternative raw material to replace starch or glucose for lactic acid production. Use of a continuous rather than a batch process [1] and optimization of various aspects of the production processes could potentially produce higher lactic acid yields. For example, supplementation with other agricultural wastes rich in protein may extend the logarithmic growth phase and increase cell number that could enhance output [34]. Further more, L.casei can produce lactic acid throughout the stationary growth phase, so this cell line is a good candidate for perfusion technology. Although improvements are feasible, this study demonstrates that production of lactic acid (a value added product) is possible via batch fermentation of sugar generated by acid hydrolysis TKP.

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REFERENCES

[1] Litchfield, J. H. et al. "Microbiological production of lactic acid". Advances in Applied Microbiology, 42, pp. 45-95. (1996)

[2] John RP, Madhavan NK, Pandey A., "Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives". Applied Microbiology and Biotechnology, 74, pp. 524–534, (2007).

[3] Pyung Cheon Lee, Woo Gi Lee, Sang Yup Lee, Ho Nam Chang, Yong Keun Chang, "Fermentative production of succinic acid from glucose and corn steep liquor by Anaerobiospirillum succiniciproducens". Biotechnol progress Engineering,5, pp. 379-381,(2000).

[4] Christina Åkerberg, Guido Zacchi, "An economic evaluation of the fermentative production of lactic acid from wheat flour". Bioresource Technology, 7, pp. 119-121.(2001)

[5] Datta R, Henry M; J; "Lactic acid: recent advances in products, processes and technologies – A-review". Journal of Chemical Technology and Biotechnology, 81, pp. 1119-1129. (2006).

[6] Global Industry Analysis, Inc. "Lactic Acid-Global Strategic Business Report", 2012 .http://www.researchandmarkets.com/reports/354880/ lactic_acid_global_ strategic_business_ report, accessed 6.12.(2013)

[7] Abdel-Rahman, Y. Tashiro, K. Sonomoto, "Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits", Journal of Biotechnology, 156, pp. 286–301, (2011).

[8] Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G, Jain AP, "Tamarindus indica: Extent of explored potential.; Pharmacognosy Reviews."5(9), pp.73-81.(2011 Jan)

[9] K.V. Peter; Handbook of Herbs and Spices, Volume 1 (Woodhead Publishing Series in Food Science, Technology and Nutrition), 2012.

[10] Ludo Cuyvers, Onvaree Assawaphanichkul, Tanuttra Ekasittipol, Sunun Laphasuk, Patthida Mhuensai, Market Profile: "Thailands export potential of tamarinds to the USA" : ASEAN Business Case Studies No 33, p. 6, ISSN-2031-4027,(August 2013)

[11] Shankaracharya N.B., Tamarind - Chemistry, technology and uses - A critical appraisal.; Journal of Food Science and Technology-Mysore 35(3), pp. 193-208, (1998)

[12] R. Chatterjee, K. Majumder, S. Sengupta, "Tamarind kernel powder co-induces xylanase and cellulase production during submerged fermentation of Termitomyces clypeatus". Biotechnology and Bioprocess Engineering, 15, pp. 845-861,(2010).

[13] Thongchul, Nuttha; Navankasattusas, Surapong; Yang, Shang-Tian, "Production of lactic acid and ethanol by Rhizopusoryzae integrated with cassava pulp hydrolysis" Bioprocess and Biosystems Engineering, 33, pp. 407-416.(2010)

[14] DuBois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F; "Colorimetric method for determination of sugars and related substances" Analytical Chemistry, 28(3), pp. 350–356.(1956)

[15] G. L. Miller; "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar". Analytical Chemistry,31 (3), pp. 426–428.(1959)

[16] Ronald Prior, Xianli Wu, Karen Schaich; "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements". Journal of Agricultural Food Chemistry 53, pp.4290−4302 (2005)

[17] Association of Official Analytical Chemist : Official methods of analysis, food analysis.15th ed. AOAC, Aspen Publisher , Washington D.C. (1990)

[18] Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I; "Determination insoluble, soluble and total dietary fibre in food products". Journal of the Association of Official Analytical Chemists (JAOAC) .;71(5), pp.1017-23.(1988 Sep-Oct)

[19] Mira Kordi, Krape, Veronika Abram, MilicaKaand Stanka Ferjan, "Determination of organic acids in white wines by RP-HPLC", Food technology and biotechnology 39(2), pp. 93–99. (2001)

[20] Dheeraj Singh, LobsangWangchu and Surendra Kumar Moond; "Processed product of Tamarind", Natural product radiance vol. 6 (4), pp. 317.(2007)

[21] G. Bustos, A.B. Moldes, J.M. Cruz, J.M. Domínguez; " Production of fermentable media from vine-trimming wastes and bioconversion into lactic acid by Lactobacillus pentosus", Journal of the Science and Food Agriculture, 84, pp. 2105–2112.(2004)

[22] Elizabeth Caplicec, Gerald F. Fitzgeralda; "Food fermentations: role of microorganisms in food production and Preservation", International Journal of Food Microbiology,50, pp.131–149.(1999)

[23] Jin, Z.T., Zhang, H.Q., Boyd, G; "Incorporation of preservatives in polylactic acid films for inactivating E. coli O157:H7 and extending microbiological shelf-life of strawberry puree", Journal of Food Protection, 73(5), pp.812-818. (2010)

[24] Calabia B P and TokiwaY; "Production of D-lactic acid from sugarcane molasses, sugarcane juice and sugar beet juice by Lactobacillus delbrueckii", Biotechnology Letters, 29, pp.1329–1332.(2007)

[25] Nakano S, Ugwu CU, Tokiwa Y; " Efficient production of D-(-)-lactic acid from broken rice by Lactobacillus delbrueckii using $Ca(OH)_2$ as a neutralizing agent", Bioresource Technology, 104, pp.791–79428. (2012)

[26] Thapa LP, Lee SJ, Yang XG, Yoo HY, Kim SB, Park C, Kim SW; "Co-fermentation of carbon sources by Enterobacteraerogenes ATCC 29007 to enhance the production of bioethanol", Bioprocess and Biosystem Engineering, 37(6), pp.1073-84.(2014)

[27] Rivas, B., Torrado, A., Rivas, S., Moldes, A. B., & Domınguez, J. M.; "Simultaneous lactic acid and xylitol production from vine trimming wastes ", Journal of the Science of Food and Agriculture, 87(8), pp. 1603-1612.(2007)

[28] Yun, J.S., Ryu, H.W; "Lactic acid production and carbon catabolite repression from single and mixed sugars using Enterococcus faecalisRKY1", Process Biochemistry, 37, pp. 235–240.(2001)

[29] Kenji Okano, Shogo Yoshida, Ryosuke Yamada, Tsutomu Tanaka, Chiaki Ogino, Hideki Fukuda, Akihiko Kondo; "Improved production of homo-d-lactic acid via xylose fermentation by introduction of xylose assimilation genes and redirection of the phosphoketolase pathway to the pentose phosphate pathway in l-lactate dehydrogenase gene-deficient Lactobacillus plantarum", Applied Environmental Microbiology, 75, pp. 7858-7861.(2009)

[30] Taniguchi, M., Tokunaga, T., Horiuchi, K., Hoshino, K., Sakai, K. and Tanaka, T; "Production of L-lactic acid from a mixture of xylose and glucose by co-cultivation of lactic acid bacteria", Applied Microbiology and Biotechnology, 66, pp.160–165. (2004)

[31] Ilmen, M., K. Koivuranta, L. Ruohonen, P. Suominen, and M. Penttila; "Efficient production of L-lactic acid from xylose by Pichia stipites", Applied Environmental Microbiology, 73, pp. 117-123. (2007)

[32] Mohamed Ali Abdel-Rahman, Yukihiro Tashiro, Kenji Sonomoto; "Recent advances in lactic acid production by microbial fermentation processes", Biotechnology Advances, 31, pp. 877–902. (2013)

[33] Gao C., Ma, C., & Xu, P; "Biotechnological routes based on lactic acid production from biomass", Biotechnology Advances, 29 (6), pp. 930-939.(2011)

[34] Altaf M, Naveena B.J., Reddy G; "Use of inexpensive nitrogen sources and starch for L-(+)-lactic acid production in anaerobic submerged fermentation", Bioresource Technology, 98, pp. 498-503.(2007)