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Nguyen Phuoc Minh
Tra Vinh University, Vietnam

Investigation of lactic acid fermentation from corn by-product using *l. casei* and *L. plantarum* strain

Nguyen Phuoc Minh

Abstract
Corn by-product raw materials are then collected in that moisture is 12.6%, 44% of cellulose, lignin content 11.2% is pretty good raw material for making substrate for lactic acid fermentation. The process of hydrolysis of corn by-product is conducted by $H2SO_4$ 15%, at 121 ^{0}C, during the 90 minutes, collecting the relatively high glucose content of 3.7% (g/100 ml), for lactic fermentation. In the type 2 strain of bacteria introduced into selected research, *L. casei* and *L. plantarum*, *L. plantarum* is best suited for lactic fermentation because the strain has a pH of fermentation environment MRS = 4.25 lower strain *L. Casei* have pH= 4.75. Strain *L. plantarum* also for lactic acid concentration is 12.83 g/l higher payroll function that lactic acid strain *L. casei* obtained is 11.64 g/l. Upset after hydrolysis process could not be reused by for the low hydrolysis. Determine the optimal technology factors for lactic acid fermentation at the same rate of 6%, 5% glucose concentration, pH=6, temperature 38 ^{o}C, while lactic acid concentration obtained after 84 h is 19.07 g/l. When conducting tests of purified lactic acid from fermented fluids with the specifications that are referenced from the pilot study, the result of lactic acid purification is 51.81 g/l.

Keywords: corn by-product, lactic acid, *L. casei*, *L. plantarum*, fermentation

1. Introduction
Lactic acid is an organic acid is colorless, mild taste, is formed by the natural fermentation in products such as cheese, yogurt, soy sauce, meat and vegetables sour. Lactic acid is many applications in the food industry, light industry and in healthcare. In medicine and Pharmacology, lactic acid to cure intestinal diseases, orthopedic surgery, dental applications, lactac calcium is calcium supplements in the form of easily absorbed by the body. In light industry, lactic acid used as industrial solvents in the manufacture of paints, varnishes, dyeing and tanning. In recent years, awareness of environmental issues has been enhanced, current research trends is that the scientists are gathering to working out the kind of material that is capable of completely degradable in the natural environmental conditions after the expiry date. A series of new materials are discovered, studied and put into practical application, among which noteworthy one of polymers which are the polylactic acid (PLA). PLA is a type of polyester plastics in the straight circuit, the product of the process is identical to stop lactic acid, a type of raw material are some big production from starch (tapioca, corn, etc.), molasses by fermentation or synthesized through the process of the goods. The PLA was considered an appropriate choice to replace plastics derived from oil because it potentially degradable and low toxicity.
Lactic acid is produced from carbon-rich substrate such as glucose, milk, molasses... from many different materials, waste and agriculture being the researchers are concerned because it is a source of immense and cheap scrap, such as jackfruit, straw, cassava residue, corn flakes, wheat bran, molasses, bagasse, corn by-product..., lactic acid is used as a feedstock in fermentation technology, to devalue the product. Lactic acid actually mean not only economically but also in life.
Corn is an important food source of man and is the main food source in livestock, in addition to corn as fresh food, nutrient-rich meet for daily consumption by humans. In our country in recent years, corn acreage has the change in positive, productive corn constantly rising so the yield of corn is also constantly increased. Tra Vinh is now one of the largest hybrid corn yield, water with a total area of corn annually about 120,000 hectares and the output reached over 520,000 tons of maize. Tentatively, the next time the Tra Vinh will increase corn acreage to around 140,000 hectares with an output of about 600,000 tons and becoming the most corn

Correspondence:
Nguyen Phuoc Minh
Tra Vinh University, Vietnam

yield and water so this is also the source of corn by-product emitted a lot. At present, the amount of corn by-product emitted largely making use of high value to the harvest, the farmer just released by burning materials right on the field created the harmful toxins such as CO_2, dust etc. This causes enormous environmental pollution and waste materials are derived from this plant to make the corn by-product in the manufacture of very large meaning for life, has just increased economic value at the same time solved the problem is pollution problem today. By-product of corn with the main ingredient is cellulose, hemiceluloza-mainly pentosan, and lignin, corn by-product offers high xenlulozo content to after the hydrolysis conditions for lactic acid fermentation.

There are several outstanding studies mentioned to lactic acid fermentation from corn or corn by-product. P. Mercier *et al*. (1992) showed the kinetics of lactic acid fermentation on glucose and corn by *Lactobacillus amylophilus*. The biosynthesis of ammonium lactate, a product of lactic acid fermentation was studied from corn and glucose at five different pH values of 5.4 to 7.8. In the glucose fermentations, a 100% conversion of substrate was obtained resulting in a maximum lactic acid production yield of 93.2%. The optimum pH for the maximum volumetric rate of lactic acid biosynthesis (1.56 g dm^{-3} h^{-1}) was between 6.0 and 6.5. The corn fermentations were slower than the glucose fermentations with a resulting lactic acid yield of 67.5%. Hydrolysis of corn by enzymatic or chemical methods as well as the use of ammonium hydroxide for pH control increased both the final concentration and the rates of lactic acid production. An enhanced yield of more than 90% was finally obtained in the corn fermentations. A logistic model adequately described the kinetics of biomass growth, lactic acid production and glucose utilization in the glucose fermentations at different pH values. The dynamics of lactic acid formation in the corn fermentations were also successfully described by the developed model. The dependence of the model parameters on pH was investigated.

Ji Yan *et al*. (2001) investigated the lactic acid fermentation from enzyme-thinned starch with immobilized *Lactobacillus amylovorus*. Immobilized *Lactobacillus amylovorus* converts enzyme-thinned starch (ETS) to lactic acid. ETS was gelatinized and thinned with -amylase. The standard medium contained w = 3 % (w/v) Yeast Extract, ETS and j = 10 % (v/v) Friedman's mineral solution. As in free-cell fermentation, increasing levels of yeast extract resulted in faster production rates and higher cell numbers in alginate beads but not in increased productivity per cell. In immobilized-cell batch fermentation, the yeast extract requirement could be reduced to 0.75%. Lactic acid productivity could be maintained in repeated batch fermentations.

C. Ruengruglikit and Y.D. Hang (2003) examined L(+)-Lactic acid production from corncobs by Rhizopus oryzae NRRL-395. Corncobs were used as a substrate for production of L(+)-lactic acid by *Rhizopus oryzae* NRRL-395. The concentration of $CaCO_3$, corncobs and Rapidase Pomaliq, a commercial apple juice processing enzyme preparation were found to significantly enhance lactic acid production by the mold. Under optimal conditions (0.2 g/100 mL $CaCO_3$, 0.5 mL/100 mL Rapidase Pomaliq, and 5 g/100 mL corncobs), *R. oryzae* NRRL-395 yielded 299.4±6.8 g per kg dry matter of corncobs after 48 h of fermentation at 30 °C.

Beatriz Rivas *et al*. (2004) studied lactic acid production from corn cobs by simultaneous saccharification and fermentation: a mathematical interpretation. Milled corn cob samples were treated with water (autohydrolysis treatment) under optimised operational conditions to cause the selective depolymerisation of hemicelluloses, and the cellulose-enriched residue was used as a substrate for lactic acid production by simultaneous saccharification and fermentation (SSF) in media containing cheap nutrients (spent yeast cells and corn steep liquor (CSL)), cellulolytic enzymes and *Lactobacillus rhamnosus* CECT-288 cells. SSF experiments were carried out at 45 °C under a variety of operational conditions (enzyme to substrate ratios in the range 5–55 FPU/g substrate, liquor to solid ratios in the range 6–10 kg/kg and spent yeast cells concentration in the range 5–20 g/L). In the initial SSF stages, cellobiose and glucose accumulate in the reaction media, and then decreased. The cellulose conversion into lactic acid obtained at the end of experiments was modelled by means of an empirical model. For process times longer than 25 h, cellulose hydrolysis became the rate limiting step of the SSF process, and the lactic acid concentration profiles followed a typical hyperbolic behaviour. Based on this finding, a simple model able to reproduce the time course of lactic acid was developed.

Niju Narayanan *et al*. (2004) demonstrated L (+) lactic acid fermentation and its product polymerization. The microorganisms being used for lactic acid fermentation, the raw materials reported, the various novel fermentation processes and its processing methods have been reviewed. The properties and applications of lactic acid, its derivatives and polymer have been discussed. The various routes to polymerization and the companies presently involved in lactic acid production have been covered.

Shigenobu Miura *et al*. (2004) produced L-lactic acid from corncob. The optimum temperature, initial pH, amount of added enzyme and substrate (corncob) for the hydrolysis of corncob by *Acremonium cellulase* were 35 °C, 4.5, 10 u/g-corncob and 100 g/l, respectively. Under the optimum conditions, more than 55 g/l of reducing glucoses were hydrolyzed from 100 g/l of corncob to 34 g/l of glucose and 12 g/l of xylose based on dried corncob. More than 25 g/l of L-lactic acid was produced from this enzymatic hydrolyzate and less than 5 g/l of xylose remained in the 3-l airlift bioreactor. The production of L-lactic acid by simultaneous saccharification and fermentation (SSF) was also carried out in the 3-l airlift bioreactor using *Acremonium thermophilum* (cellulose-producer) and *Rhizopus sp*. MK-96-1196 (lactic acid-producer). More than 24 g/l of L-lactic acid was produced from 100 g/l of untreated raw corncob.

Hurok Oh *et al*. (2005) experimented lactic acid production from agricultural resources as cheap raw materials. Agricultural resources such as barley, wheat, and corn were hydrolyzed by commercial amylolytic enzymes and fermented into lactic acid by Enterococcus faecalis RKY1. Although no additional nutrients were supplemented to those resources, lactic acid productivities were obtained at >0.8 g/l h from barley and wheat. When 200 g/l of whole wheat flour was hydrolyzed by amylolytic enzymes after the pre-treatment with 0.3% (v/v) sulfuric acid and sterilized by filtration, E. faecalis RKY1 efficiently produced lactic acid with 2.6 g/l h of lactic acid productivity and 5.90 g/l of maximal dry cell weight without additional nutrients. Lactic acid productivity and cell growth could be enhanced to 31% and 12% higher values than those of non-adapted RKY1, by adaptation of E. faecalis RKY1 to CSL-based medium. When the medium contained 200 g/l of whole wheat flour hydrolyzate, 15 g/l of corn steep liquor, and 1.5 g/l of yeast extract, lactic acid productivity and maximal dry cell weight were obtained at 5.36 g/l h and 14.08 g/l, respectively. This result represented an improvement of up to 106% of lactic acid productivity and 138% of maximal dry cell weight in comparison to the fermentation from whole wheat flour hydrolyzate only.

Kenji Okano, *et al*. (2009) investigated efficient production of optically pure d-lactic acid from raw corn starch by using a genetically modified-lactate dehydrogenase gene-deficient and α-amylase-secreting *Lactobacillus plantarum* strain. In order to achieve direct and efficient fermentation of optically pure D-lactic acid from raw corn starch, they constructed L-lactate dehydrogenase gene (ldhL1)-deficient *Lactobacillus plantarum* and introduced a plasmid encoding Streptococcus bovis 148 α-amylase (AmyA). The resulting strain produced only D-lactic acid from glucose and successfully expressed *amyA*. With the aid of secreting *AmyA*, direct D-lactic acid fermentation from raw corn starch was accomplished. After 48 h of fermentation, 73.2 g/liter of lactic acid was produced with a high yield (0.85 g per g of consumed glucose) and an optical purity of 99.6%. Moreover, a strain replacing the ldhL1 gene with an *amyA*-secreting expression cassette was constructed. Using this strain, direct D-lactic acid fermentation from raw corn starch was accomplished in the absence of selective pressure by antibiotics. This is the first report of direct D-lactic acid fermentation from raw starch.

Zulfiqar Ali *et al*. (2009) described production of lactic acid from corn cobs hydrolysate through fermentation by Lactobacillus delbrueckii. This study described several essential factors for indirect and effective lactic acid production from food wastes by strains of Lactobacillus delbrukii using corn cob hydrolysates. The fermentation conditions considered were glucose concentration (1 - 5%), temperature (34 – 40 ^{0}C), time (0 - 8 days) and pH (5 - 6). After pretreatment of corn cobs by dilute acid, the lignocellulosic residue was used as raw materials for the saccharification and followed by lactic acid fermentation. The best fermentation conditions were 40 ^{o}C temperature, after 84 h of fermentation time. In the saccharification and subsequently fermentation process, the final concentration of lactic acid reached 17.73 g/L based on the glucose (reducing glucoses) extracted from the saccharified corn cobs at pH 5-6.

Cristian J. B. de Lima *et al*. (2010) carried out L(+) lactic acid production by new *Lactobacillus rhamnosus* B 103. L(+) Lactic acid fermentation was studied by *Lactobacillus rhamnosus* sp. under the effects of pH control and a low cost nutritional medium (glucose cane juice and corn steep liquor-CSL). Central composite design (CCD) was employed to determine maximum lactic acid production at optimum values for process variables and a satisfactory fi t model was realized. Statistical analysis of the results showed that the linear and quadratic terms of two variables (glucose cane juice and pH) had significant effects. The interactions between the three variables were found to contribute to the response at a significant level. A second-order polynomial regression model estimated that the maximum lactic acid production of 86.36 g/L was obtained when the optimum values of sucrose, CSL and pH were 112.65 g/L, 29.88 g/L and 6.2, respectively. Verification of the optimization showed that L(+) lactic acid production was of 85.06 g/L. Under these conditions, YP/S and QP values of 0.85 g/g and 1.77 g/L/h, respectively, were obtained after 48 h fermentation, with a maximal productivity of 2.2 g/L/h at 30 h of process.

Guo Y *et al*. (2010) determined efficient production of lactic acid from sucrose and corncob hydrolysate by a newly isolated Rhizopus oryzae GY18. The aim of this study was to investigate production of L-lactic acid from sucrose and corncob hydrolysate by the newly isolated *R. oryzae* GY18. *R. oryzae* GY18 was capable of utilizing sucrose as a sole source, producing 97.5 g l(-1) L-lactic acid from 120 g l(-1) sucrose. In addition, the strain was also efficiently able to utilize glucose and/or xylose to produce high yields of L-lactic acid. It was capable of producing up to 115 and 54.2 g l(-1) lactic acid with yields of up to 0.81 g g(-1) glucose and 0.90 g g(-1) xylose, respectively. Corncob hydrolysates obtained by dilute acid hydrolysis and enzymatic hydrolysis of the cellulose-enriched residue were used for lactic acid production by *R. oryzae* GY18. A yield of 355 g lactic acid per kg corncobs was obtained after 72 h incubation. Therefore, sucrose and corncobs could serve as potential sources of raw materials for efficient production of lactic acid by *R. oryzae* GY18.

Wang L *et al*. (2010) carried out efficient production of L-lactic acid from corncob molasses, a waste by-product in xylitol production, by a newly isolated xylose utilizing *Bacillus sp*. strain. Corncob molasses containing a high content of xylose, which is one of the lignocellulosic biomasses and a waste by-product from xylitol production, was used for L-lactic acid production via a newly isolated xylose utilizing *Bacillus sp*. strain XZL9. *Bacillus sp*. strain XZL9 can utilize the mixture of glucoses including xylose, arabinose, and glucose in corncob molasses for L-lactic acid production. High concentration of L-lactic acid (74.7 g l^{-1}) was obtained from corncob molasses (initial total glucoses of 91.4 g l^{-1}) in fed-batch fermentation. This study provides an encouraging means of producing L-lactic acid from lignocellulosic resource such as the low-cost corncob molasses.

Cristina Sánchez *et al*. (2012) examined lactic acid production by alkaline hydrothermal treatment of corn cobs. An experimental study was carried out for the corn cobs thermal conversion to obtain the maximum content in lactic acid. For this purpose, under the same conditions (275 °C and 30 min) different concentration of $Ca(OH)_2$ as alkaline catalyst were used (from 0.32 M to 1 M). The maximum content of lactic acid (6.72 ± 0.31 g/L) was obtained with 0.7 M of $Ca(OH)_2$. With this catalyst concentration, different reaction conditions were used (250, 275 and 300 °C and 15, 30 and 45 min). The optimal conditions to produce the highest yield of lactic acid from corn cobs in alkaline conditions were determined at 300 °C and 30 min, achieving 44.76 ± 2.59% respect to the total cellulose and hemicellulose contained in the initial corn cobs (7.38 ± 0.43 g/L of lactic acid).

Zhangwei Xue *et al*. (2012) showed efficient production of polymer-grade L-lactic acid from corn stover hydrolyzate by thermophilic *Bacillus sp*. strain XZL4. Lactic acid has been identified as one of the top 30 potential building-block chemicals from biomass. Therefore, the search for cheap raw materials is an objective to reduce the production costs. Efficient polymer-grade L-lactic acid production was achieved in this report by a thermophilic strain *Bacillus sp*. XZL4 using corn stover hydrolyzate as sole carbon source. High L-lactic acid concentration (81.0 g L-1) was obtained from 162.5 g L-1 concentrated corn stover hydrolyzate (total reducing glucose of 83.0 g L-1) with a volumetric productivity of 1.86 g L-1h-1 (0–36 h) and a product yield of 0.98 g g-1 total reducing glucoses. This is the highest L-lactic acid concentration and yield reported from corn stover hydrolyzate. And the high optical purity of L-lactic acid obtained in this study also indicated that *Bacillus sp*. XZL4 is a promising polymer-grade L-lactic-acid producer from cellulosic biomass.

At present, the use of by-products and wastes in production agriculture for our country is still very new and is the direction being the pick, in which lactic acid fermentation technology from agricultural wastes is a new trend to help receive the desired product and resolve environmental problems. So we decided to choose the subject *"Investigation of acid lactic fermentation from corn by-product using L. Casei and L. plantarum strain"*.

2. Material & Method

2.1 Material

Use corn by-product was the purchase in the households in the province of Hau Giang. Lactic acid fermentation from hydrolysis of corn by-product in laboratory scale.

2.2 Research method

Before conducting the study of lactic acid fermentation from corn by-product, we proceed to identify a number of chemical composition of corn by-product such as cellulose, lignin, moisture etc. Corn by-product after it determined the chemical composition will be giving away money handled in aqueous $H2SO_4$ 0.5%, for 30 minutes. Later went to determine the amount of cellulose, lignin and moisture.

Corn by-product once dealt with will be put away hydrolysis with $H2SO_4$ 15%. At the rate of 1: 10 (w/v), at 121 ^oC, 90 minute period. After hydrolysis then neutralize NaOH 15% filter separates the excess our receiving are fluided after hydrolysis. After obtaining room hydrolysis we proceed to determine the reducing glucoses content of the hydrolysis by color comparison method using dinitrosalicylic acid (DNS). To determine the concentration of glucose we proceeded to build the standard glucose to from the rear, we calculate the concentration are based on the standard line equation.

Strain breeding of bacteria after cultivation of breeding lines activated level 1 then proceed up yeast in the environment 2: 1) proceed to inoculate 2 strain of bacteria into the environment MRS liquid at ambient temperature 37 ^oC, 48 hours to determine the capacity of the fermentation strain of bacteria by measuring the pH of fermentation environment. 2) conduct 2 fermentation strain of bacteria into the room environment hydrolysis of corn by-product concentration 5% glucose has nutritional ingredients supplements and cleaning sterilization. Fermentation is performed in conditions of temperature 37 ^oC, 48-hour period, pH = 6. Then proceed to determine the amount of lactic acid forms to determine the capacity of the fermentation strain bacteria.

3. Result & Discussion

3.1 Survey chemical composition of corn by-product before pre-treatment

Table 1: Chemical composition of Corn by-product before pre-treatment

Composition	Percentage %
Cenlulose	44%
Lignin	11.2%
Moisture	12.6%

From the results shown in table 1, corn by-product material contains cellulose quite high compared to the overall rate of corn by-product in the world. Concentration of cellulose will directly influence the concentration obtained after the course of hydrolysis of cellulose content, the higher the glucose content of as much. Lignin content of 11.2%, compared to the amount of lignin in the corn by-product world 6.7-24.5%. As such, corn by-product material could be viewed as potential source material for the production of lactic acid.

3.2 Survey Chemical composition of Corn by-product after pre-treatment

Table 2; Chemical composition of Corn by-product after pre-treatment

Composition	Percentage %
Cenlulose	74
Lignin	10.8
Moisture	8

Results indicate% of cellulose have changed significantly compared to the raw material, the process has to dissolve other compounds such as lignin, cellulose conversion to hemiceluloza, lignin content changes are not significant. Moisture content of corn by-product reduction after the pretreatment process by degrading water retention of the structures in the corn by-product, this creates favorable conditions for the process of hydrolysis of the latter. So we proceeded to treat the entire corn by-product materials according to the above methods to cater to the next study.

3.3 Hydrolization of corn by-product

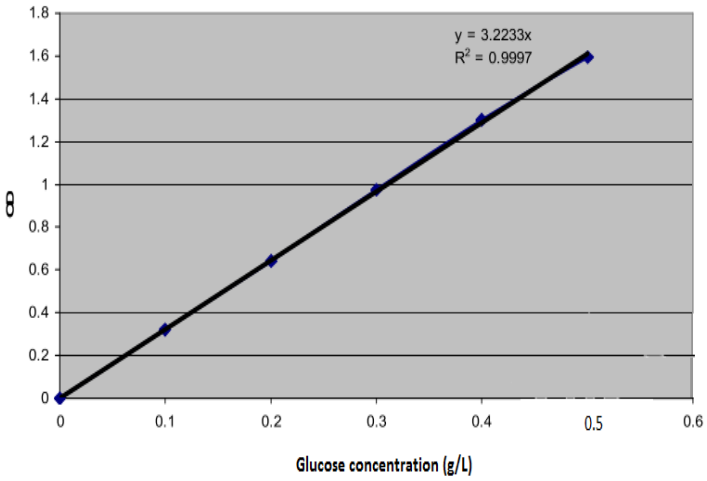

Fig 1: Hydrolization of corn by-product

Based on a standard line equation and results measured OD of the hydrolysis ofwe determined the concentration of the hydrolysis. The results measuring the concentration of glucose 3 samples are shown in table 3 below:

Table 3: Glucose concentration after hydrolization

Sample	OD (x)	Glucose concentration g/l, y = 3.2233x
1	1.253	38.87
2	1.199	37.19
3	1.178	36.54
Mean		37.53

So, to fluid our hydrolysis is obtained that represents the fluidd path is 37.53 g/l, of the performance of process of hydrolysis was 74.04% compared with his work in the world, our glucose obtained is quite high.

*** Chemical composition in waste:** After hydrolysis, residue collected on filter paper is then washed, dried to constant mass brought to take weight, with 50 g substrate corn by-product after hydrolysis obtained 12.8g residue. We then proceeded to determine the amount of cellulose and moisture. The results are shown in table 4.

Table 4: Chemical composition in waste

Composition	Percentage (%)
Moisture content	8%
Xenluloza	34%

With the results shown in table 4 it is shown, in the cellulose content of pulp is also quite high so we went to survey the process of hydrolysis of cellulose in upset to see the performance of reuse residue. The process of hydrolysis was carried out 10 g excess in 100 ml $H2SO^4$ 15%, hydrolysis of 121 ^oC temperatures, time of 90 minutes. The results obtained the reducing glucose concentration glucose epidemic is 3.04 g/l, the reducing glucose concentration is very low compared to the amount of cellulose in excess earnings. According to the results of research in group 4 and some studies make use of the excess corn after the hydrolysis of cellulose component is mostly hard to hydrolysis and some other components. Excessive intake should not be re-used that it can only be used for other purposes are less economic value.

3.4 Strain prepraration for lactic fermentation

3.4.1 Activation of bacteria strain

Environment for *Lactobacillus casei* bacteria cultivation and the bacteria *Lactobacillus plantarum* is the environment MRS agar by Merck of Germany. This is the environment that make available. Strain bacteria *Lactobacillus casei* and *Lactobacillus plantarum* strain in freeze-dried form, animation in the environment. Chain transplants 2 levels and keep plummeting temperatures. The purpose creates favourable conditions for the fermentation process.

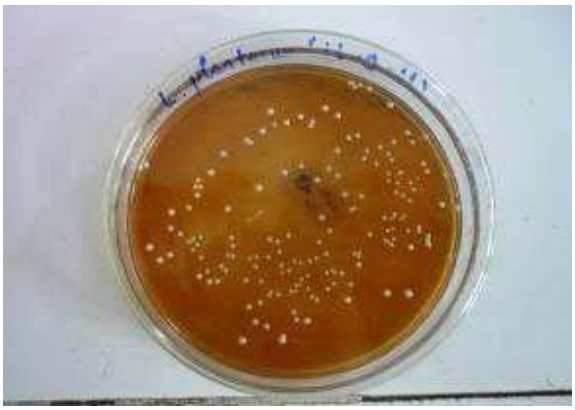

Fig 2: *L. plantarum* activated

Fig 3: *L. casei* activated

3.4.2 Selection of bacteria strain for lactic fermentation

Table 5: pH of MRS fermentation for two strain *Lactobacillus plantarum* and *Lactobacillus casei*

MRS medium	*L. plantarum*	*L. casei*
pH	4.25	4.75

Table 6: Lactic acid formation from *L. plantarum* and *L. casei fermented* in hydrolyzation medium

MRS medium	*L. plantarum*	*L. casei*
Lactic acid concentration (g/L)	12.83	11.64

Through the results table 5 and 6 showed the capacity born of lactic acid strain *L. plantarum* higher strain L. casei. The results of table 5, we find the pH of fermentation environmental strain bacteria *L. plantarum* has pH = 4.25 lower pH of fermentation environment strain bacteria L. casei have pH = 4.75. This demonstrates the environment of fermentation of the strain of bacteria *L. plantarum* to produce more lactic acid fermentation environment of strain L. casei. The results of table 6, we found the bacteria *L. plantarum* for lactic acid concentration is 12.83 g/l higher lactic acid concentration to which the bacteria L. casei born is 11.64 g/l when fermented in fluid environment hydrolysis of corn by-product. This shows both the strain of bacteria are capable of living lactic acid in room hydrolysis of corn by-product, so fluid environment hydrolysis of corn by-product is also a good environment for the lactic fermentation. However, the bacteria *L. plantarum* produces lactic acid concentration is higher so we decided on the strain of bacteria *L. plantarum* as the strain of bacteria to lactic acid fermentation.

3.4.3 Breeding bacteria strain

3.4.3.1 Culturing and breeding bacteria strain *Lactobacillus plantarum*

Strain bacteria *L. plantarum* after activation will be conducting breeding level 1 in the environment MRS 30 ^oC temperature, time of 48 hours, then go preserved in temperature 0 ^oC to cater for research. After breeding level 1, we conducted bred 2 supply strain bacteria *L. plantarum* in the fluid environment hydrolysis of corn by-product. This breeding process aimed at creating conditions for gradually adapted bacteria strain with fermentation environments facilitate fermentation to reach more effectively.

3.4.3.2 Determination of the number of cells of microorganisms in fermented medium:

In the study, in response to the number of cells of bacteria for fermentation, we proceed to determine the number of cells of bacteria in fermenting indirectly by specifying the number of bacteria through quantitative methods of cells by optical density measurement method.

Proceed to dilute the bacteria were cultured in fermentation environment in distilled water at many different dilution degrees into degrees of dilution of degree 10 in a row such that the dilution and bacterial cell densities appropriate to appear the individual contact surface bacterial fossils. Proceed to count the number of bacteria on agar plates contact inferred the number of cells/ml of cultured fluid.

Over 48 hours to end the process of incubating in 30 ^oC, we count the number of stray bacteria grow on the agar plates. At

the level of dilution of 107 on 2 petri we count bacteria tribe grows on 2 disks in the order once the disc is: 86 and 102. We determined the density of cells strain bacteria *L. plantarum* in the medium level of dilution of 107 is I=9.4x10^9 CFU/ml. This result ensures effective fermentation because I > 1.3x10^9 CFU/ml. results of the measured OD corresponds to the number of cells counted in each different dilution levels are presented in table 7.

Table 7: OD at cell density of *L. plantarum*

No	Dilution	OD	Cell density (CFU/ml)
1	10^4	0.361	…..
2	10^5	0.218	…….
3	10^6	0.187	2.62 x 10^9
4	10^7	0.139	9.4 x 10^9
5	10^8	0.098	15 x 10^9

From the results in table 7, we proceed to build the same cell number standards hafts unleashed is the opacity (OD 620 nm), the raging shaft is the same cell number. Find the equation performed the standard form: y = a x + b y =OD 620 nm; x = the number of identical cells (CFU/ml), from the equation we can quantify the density of cells indirectly via the optical density measuring OD at 620 nm.

3.5 Factors affecting to lactic acid fermentation

Glucose medium after adjustment for the concentration of the survey 5% (g/100 ml), we conducted supplementary nutrition, followed by cleaning sterilization and fermentation

3.5.1 Effect of breeding ratio to lactic acid fermentation

Fixed 5% glucose concentration, pH = 6, temperature 37 ^oC, time 72 hours. Proceed to prepare the sample 7 experiments with different breeding rate 2-8%, density is 9.4x109 cells/ml. Experimental results are shown in figure 4.

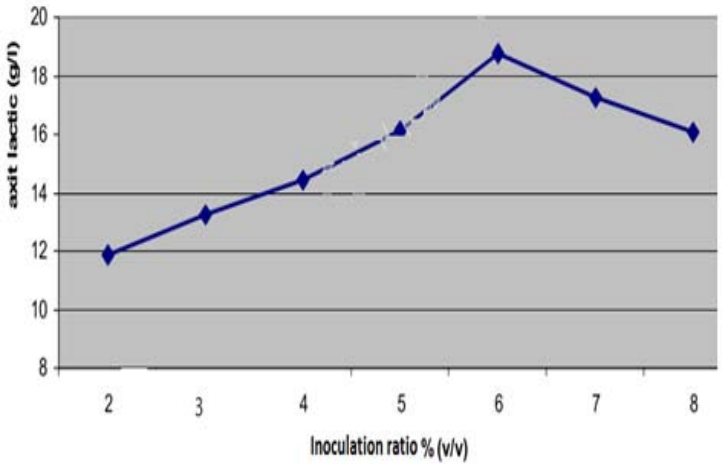

Fig 4: Effect of cell inoculation for acid lactic formation

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Through the results of figure 4 we see when the same rate increase then the lactic acid concentration also increased from 11.88-18.76 (g/l), reached the same rate at 6%, then the acid content of the highest obtained 18.76%, which means that when the same rate increase, the lactic acid concentration also increases but to a certain value then the lactic acid concentration decreased mean ratio more like falling in a certain limit will produce lactic acid, but if the pass which will reduce acid concentration. If the same rate added too little, it is not enough for fermentation, fermentation will take time, spend time and energy. In contrast, if the same rate much reduced osmotic pressure, microbial cells were inhibited simultaneously the same rate much will create nutritional competition during periods of growth, lactic acid products produced will be lower, and increases the costs for the breeding process at the same time will cause an inhibition of fermentation process produces byproducts. Finding out the same rate appropriate for the very fermentation process is necessary. Through our research findings in the same rate 6% we also obtained the highest concentration of lactic acid to approximate the author's research Shadi Bolourian (2010) has studied that *Lactobacillus plantarum* strains rate 5% for the lactic fermentation is the best.

3.5.2 Effect of glucose concentration to lactic acid fermentation

Experiments were conducted with the following conditions: additional breeding rate is 6%, pH = 6, the time of 72 hours, the temperature 37 ^oC, surveys in the different glucose concentration of 1-7%. Experimental results are shown in figure 5 below.

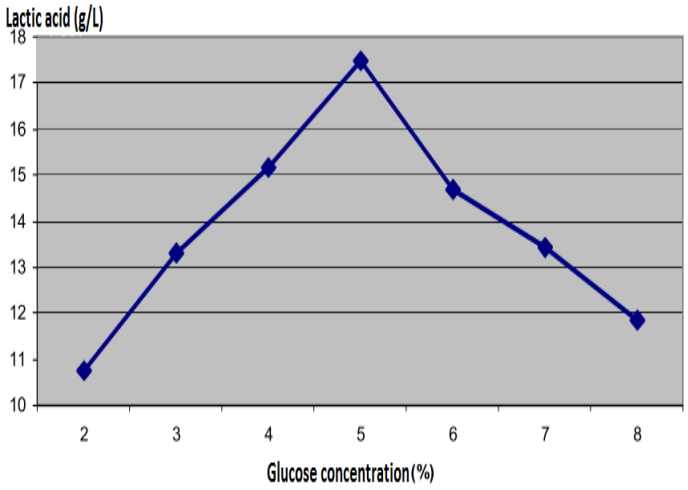

Fig 5: Effect of glucose concentration to lactic acid fermentation

Through the results figure 5, we saw increased glucose concentration, the lactic acid concentration is rising, but if the concentration of the higher lactic acid concentration decreased. When glucose concentration increased from 1-5%, lactic acid content varies from 10.74-17.50 (g/l), the concentration reaching 5% then the lactic acid content of function at the highest level, when 6-8%, lactic acid concentration decrease. This shows that when the concentration of the high street too, it is not appropriate for the active bacteria, will inhibit the process of growth and development of lactic acid bacteria. Hence, the need to adjust the glucose concentration is suitable to create favorable conditions for the fermentation process. In this study the influence of the concentration of glucose to lactic fermentation, we found in the concentration of 5% is obtained the highest concentration of lactic acid is 17.5%. This is approximate to the results of research of author Xueliang & Shen Liming Xia (2006) when research produces lactic acid from some agricultural wastes, including corn by-product, they suggested that glucose concentration of 6% was appropriate.

3.5.3 Effect of temperature to lactic acid fermentation

Experiments conducted in about 34 to 40 ^oC determine the best temperature for the fermentation process. With the conditions fixed concentration of glucose 5%, the same rate of 6%, the time of 72 hours. Experimental results are shown in figure 6.

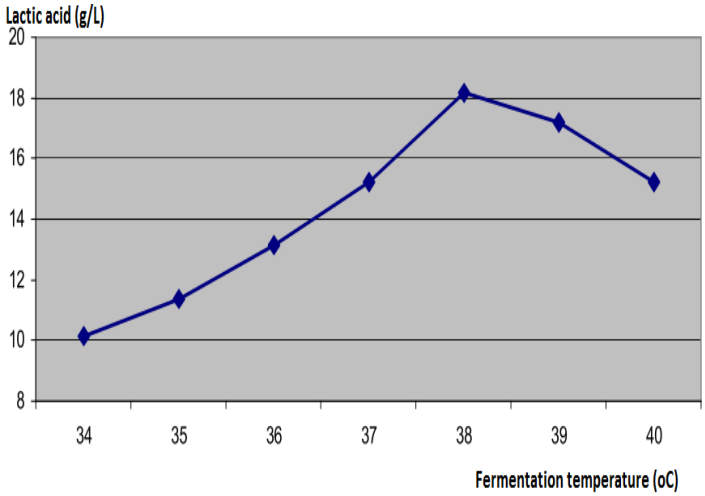

Fig 6: Effect of fermentation temperature to lactic acid formation

Plot of figure 6 illustrates, at a range of temperatures in 34 °C to 38 °C. Lactic acid concentration increases the rise of temperature, but when the temperature rises to 39 °C, then fell. With temperatures around 34-38 °C. Lactic acid amounts obtained ascending from 10.14-18.18 g/l, temperature 39-40 °C, the lactic acid concentration decreased from 18.18 g/l down to 15.22-17.08 g/l, this indicates a temperature suitable for forming lactic acid is optimal, this temperature lactic acid concentration is highest. In our study, the proper temperature to that to which produces lactic acid concentration as high as 38 °C. Its concentration was obtained 18.18 g/l. Concentration is also relatively high compared to the research go ahead. Compared to some foreign material that we refer to the temperature they obtained the highest concentration of lactic acid are also within about 35-39 °C.

3.5.4 Effect of time to lactic acid fermentation

The process was conducted in the same rate added 5%, pH = 6, 5% glucose concentration, the temperature of the optimal, the dates 24, 36, 48, 60, 72, 84, 96 and 108 hours. The result was and is represented in the following figure 7.

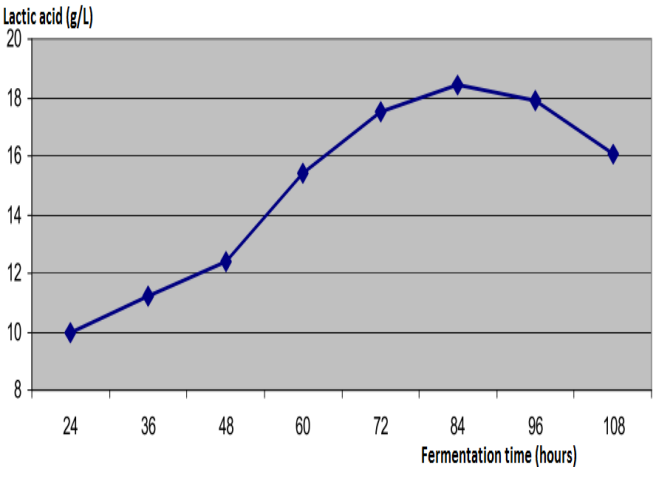

Fig 7: Effect of fermentation time to lactic acid formation

Observe figure 7 we see when time grew from 24 hours to 84 hours, lactic acid concentration also rose sharply, gaining maximum 18.43 g/l at the time 84 hours, but when time is up 96-108 and now the lactic acid concentration decreased 17.87-16.06 g/l this shows that time also affects the process of producing lactic acid.

Strain bacteria *Lactobacillus plantarum* as being put into the nutritional environment, they will use nutrition to growth and development, development process to a proper period will stop. Time to ferment fermentation performance, if too little fermentation time at this microorganism is not yet fully synthetic nutrient sources should produces less lactic acid fermentation time is too long, then after the bacteria have used up substrate to produce lactic acid, if not stop, the fermented lactic acid bacteria will continue to use lactic acid concentration created the work substrate to create extra products, this is not good for the lactic fermentation. So it's essential to find the right time to control the fermentation process to produce lactic acid content best.

3.6 Conducting the fermentation under optimal conditions

After studying the factors affecting lactic acid fermentation process, we conducted fermentation conditions optimization on to evaluate the efficiency of fermentation. Conducting experiments with the conditions: 5% glucose concentration, pH = 6, the same rate is 6%, the optimal temperature, time is 84hours. After performing experiments, we obtained the highest concentration of lactic acid 19.07 (g/l). This is fairly high concentration compared to the research in the world. According to the study by Hassan K et al. (2001) study on lactic acid production from agricultural wastes, with raw materials as corn by-product for lactic acid concentration is 20 g/l, or the study of the Zulfiqar (2009) has studied the production of lactic acid from corn by-product lactic acid function is 25.62 g/l. Research of Limin Wanga (2014) studied the lactic acid production from molasses of corn by-product, scrap of xylitol production by strain Bacillus to lactic acid concentration 26.4 g/l in general. Through the research results in the world, the lactic acid content of our study is also relatively high.

3.7 Purification of lactic acid

Through the research and reference work on refining lactic acid in countries all over the world, we conducted tests of purified lactic acid from fermented fluids are obtained. After the fermentation fluid processing to identify lactic acid content of raw, use activated charcoal 1 g/l for stripping by heat the room 15-20 minutes then filtered several times and concentrate to enhance the concentration of lactic acid is obtained.

After the lactic acid purification, we proceed to quantify levels of lactic acid obtained by the method of comparison used color para-oxydiphenyl, 575 nm wavelength measured at sample. Lactic acid concentration results we obtained were 51.81 g/l. Lactic acid levels we obtained after purification is also relatively high. However, it is still low compared with many of the world's research as the research of Zulfiqar (2009) lactic acid concentration after refining is 80 g/l, research of Madzingaidzo L. (2002) study of lactic acid purification obtained the highest concentration of lactic acid 85%. In this study, the technological conditions of refining process only references from the work world with some raw materials of similar nature. However, this refined method has been used for years in countries all over the world, because of the simple process technology with common chemicals and easy to make. Today, the more lactic acid production with the more modern method using ion exchange method, method of electrons etc because conditions are not allowed so we just made the traditional method is to use the method of precipitating acid purification effect is therefore not high, this is just considered the test course refining lactic acid from lactic acid fermented fluids from corn by-product.

4. Conclusion

This study describes several essential factors for effective lactic acid production from food wastes by strains of *L. Casei* and *L. plantarum* strain using corn by-product. Because our research is mainly on laboratory and only one subject, we should extend to other agriculture by-products in large scale; deep investigation into hydrolization of corn by-product to lactic acid fermentation. This research mainly focuses on biotransformation of xenluloza in raw materials but not investigate the effect of intermediate substances to growth of bacteria. Moreover it is necessary to perform further studies not only reduced sugar (calculated by glucose) but also sugar 5-carbon and 6-carbon.

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