

PRODUCTION AND DETECTION OF L-(+)-LACTIC ACID USING CASSAVA AS THE LOW COST FERMENTATION MEDIUM FOR THE SYNTHESIS OF BIODEGRADABLE POLYMERS AS ORTHOPEDIC DEVICES

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Abstract: Polymers based on Lactic acid are of great importance to the healthcare industry because they decompose by hydrolysis in the human body into nontoxic metabolites. Therefore, the objective of the current study was to produce L-(+)-Lactic acid using a low cost medium in order to synthesize a biodegradable polymer for healthcare industry. Powdered cassava was acid hydrolyzed using different concentration of HCl (0.5, 1, 1.5, 2, 2.5, 3, 5, 8 and 10 %) followed by a thermal treatment at 120°C for 10 minutes. Five different media (A- E) were prepared from the above hydrolyzed product along with yeast extract. *Lactobacillus casei* was inoculated to media A (Cassava- 5 g, Yeast extract-1.5 g, K₂HPO₄-0.41 g, KH₂PO₄-0.56 g), B (Cassava- 5 g, Yeast extract 1.5g, MgSO₄.7H₂SO₄- 1 g, (NH₄)₂SO₄-1 g), C (Cassava- 5 g, Yeast extract- 5g, K₂HPO₄- 2 g, NaAc- 5 g, MgSO₄.7H₂SO₄- 0.02 g, MnSO₄.4H₂O-0.05 g), D (Cassava- 5 g, Yeast extract- 1.0g, K₂HPO₄- 0.41g, MgSO₄.7H₂SO₄- 1g, (NH₄)₂SO₄-1g), and E (Cassava crumble- 5 g, Yeast extract- 1.0g, K₂HPO₄- 0.41g, MgSO₄.7H₂SO₄- 1g, (NH₄)₂SO₄-1g). Medium A, B, and C were inoculated with *L. delbrueckii*. The lactic acid produced by each species in different media were quantified using Agilent 1260 infinity HPLC. According to the Benedict test the 2 % HCl was obtained to provide efficient amount of L-(+)-lactic acid via acid hydrolysis. Medium D fermented with *L. casei* was identified as the effective low cost medium for large scale fermentation. The lactic acid concentration of the medium D was detected as 259 mg/L. Medium D was identified as effective medium for large scale fermentation for the production lactic acid with *L. casei*. Purification and polymerization need to be performed in order to synthesize the biopolymer.

Keywords: L-(+)-Lactic Acid, Cassava, *Lactobacillus casei*, *Lactobacillus delbrueckii*

Background

Many scientists who work with polymers are closely working with new inventions for devices such as instruments for medical fields. Polymer scientists have gained tremendous advances over the past 30 years, regards to their inventions. These inventions have a general criteria for selecting a polymer, in which the biomaterial selected as to match the mechanical properties and the time of degradation of the application; thus fulfil the needs of the of the invention (Middleton and Tipton, 2000).

Lactic acid based polymers have deserved prodigious attention, because of the decomposing process via hydrolysis metabolism; which in the human body is converted into nontoxic metabolites. Biodegradable polymers such as Poly L-Lactic Acid (PLLA) and Polyglycolide (PGA) are produced from renewable sources which contain sugar and starch. These polymers play important role not only in the plastics industrial and in the biopolymers for medical industry (Kaihara *et al*, 2007).

In recent years, the health care sectors use Poly Lactic Acid (PLA) in many ways. Especially the PLLA is used as a representation of the orthopedic metal implants; this is because of PLLA is more strong and harmless to human. The implant using metal plate are considered expensive and could be only removed via a surgery. Despite the main drawback, it also obtains a time period for the wound to heal properly, due to process of removing the metal implants via a surgical operation. Therefore, the metal implantation is considered as a time and energy scarification. Hence, the PLA is used as a biopolymer, which would be biodegradable and is nontoxic to humans. Another reason is that, PLA could be fermented easily using natural fermentation process, thus it is considerably low cost compared few metal implants (Agrawal *et al*, 1995).

According to Nampoothiri in his article in 2010, he has quoted that the estimated “price of PLA is ca. 2.2 \$/kg”; thus this means in an industrial production, the price of confined lactic acid ought to “be less than 0.8 \$/kg” (Nampoothiri *et al*, 2010). One of the key feature in reducing the price of production of lactic acid is by the cost of raw material; which is used for funding the microbial growth in the fermentation medium. This is also plays an important role when it comes to the growth of fastidious lactic acid bacteria (Zaunmuller *et al*, 2006 and Nampoothiri *et al*, 2010).

The PLA polymer production involves not only a chemically pure lactic acid, but also an optically pure lactic acid. The chemical purity is mainly contingent on the fermentation medium constituents; especially to provide cheap sources of sugar from raw material. Whereas, the optical purity of PLA is certified by the optical purity of lactic acid, for which the production of lactic acid is proceeded through several strains of microorganisms under optimized fermentation conditions. In contrast to other fermentation harvests, lactic acid production via monosaccharides is 90 % high (Abdel-Rahman *et al*, 2011).

The PLA provides both high melting point and crystallinity (Ghaffar *et al*, 2014). Lactic acid has two optical isomers, L-(+)- lactic acid and D-(-)- lactic acid (Narayanan *et al*, 2014). It is classified as GRAS (generally recognized as safe). The production of PLA requires high optical purity. Therefore, the properties of PLA are adjusted accordingly to a ratio of the L- and D-PLA to form the co-polymer, in which D-form increases the melting point of the copolymer. Both the optically pure L- and D- lactic acid are efficiently achieved by microbial fermentation, thus presently 95 % of industrial lactic acid production is supported by microbial fermentation process (Ghaffar *et al*, 2014).

The lactic acid production by LAB (Lactic Acid Bacteria) uses the biological pathway classified as homofermentative method, since LAB's hexose metabolism is under the non-restrictive conditions, which is entirely through the pathway of Embden-Meyerhof to pyruvate pathway; this is then used to redevelop the reducing power of NADH in the lactate dehydrogenase (LDH) catalyzed reaction to lactic acid. Nevertheless, at times such as low glycolytic flux and slow growth rate help in the formation of acetic acid, formic acid and ethanol, as well to lactic acid (Zaunmuller *et al*, 2006).

The advantage in microbial fermentation is in synthesizing one of the isomers, and producing an optically pure product by selecting a particular strain the lactic acid bacteria (LAB). Meanwhile in a synthetic production the outcome is a racemic mixture of lactic acid. The optically pure lactic acid production is essential in the synthesis of polymer construction. Additionally, due to the presence of L-lactate dehydrogenase, L-(+)-lactic acid is recycled by human metabolism (Jarvi's, 2001).

Now a days, the production of L-(+)-lactic acid is used to enhance the economics of the lactic acid fermentation process, it is used to increase the concentration of lactic acid in the medium via optimization of fermentation medium (Panesar *et al*, 2010). The present work was, therefore, carried out to optimize the medium for efficient lactose conversion in cassava powder to L-(+)-lactic acid.

The process fermentation originates from a sugar known as the substrates. Few carbohydrate such as, potato starch, corn starch, milk whey and molasses are used as the substrate for the production of lactic acid. Sugars

hydrolyzed from different substances of starch and molasses are plentifully used substrate when it comes to industrial production of lactic acid via microbial fermentation process (Fakhravar *et al*, 2012).

Nevertheless, choice of substrate is important depending upon factors such as (1) its availability in the area, (2) treatment required prior to fermentation, and (3) the processing costs. It is also noted widely, in the production of lactic acid via microbial fermentation using pure sugar is the best substrate but the purification process of sugar is most possibly an expensive process (Fakhravar *et al*, 2012). It is a main reason using cassava as the main sugar substrate in this study, as to the availability and high starch content in them.

The lactic acid production for commercial manufacture via fermentation technology is mainly influenced by the raw material used; the main important factor while using raw material depends mainly on the a cost efficiency of the raw material. Therefore, it is important to select a raw material for industrial production of lactic acid with a number of characteristics such as the following (1) low cost, (2) rapid rate of fermentation, (3) lowest amount of contaminants, (4) high yields of lactic acid production, (5) least or no formation of by-products and (6) availability for whole year (Ghaffar *et al*, 2014).

In Sri Lanka cassava is not considered as a nutritionally higher crop but due to the crop's drought resistance, relative resistance from pest attack and the ability to yield under unfavorable conditions with less requirements plus attention put in it, makes it the highest grown crop in the agriculture list. Thus, it is found throughout the island apart from high raise areas concentrated with the wet and intermediate zones of Sri Lanka. Though cassava is considered as a backyard crop, it is also carried out in large scale cultivation in open-lands of the wet zone such as Gampaha, Colombo, Kegalle, Rathnapura and Matara Districts; in intermediate zone of Kurunegala district. While large scale production also occurs in the dry zone which includes Putlam, Anuradhapura, Ampara, Hambantota, Moneragala Districts. Cassava plant production is peak during falls of Maha season. (Department of Agriculture, 2006).

Furthermore, cassava produces the highest amount per unit of area of soluble carbohydrates in its conversion from solar energy. Among all the starchy sources, the cassava gives higher production carbohydrate which is about 40 % is higher than rice and 25 % more than maize. The composition of the cassava is includes of 70 % of moisture, 24 % of starch, 2 % of fiber, 1 % of protein and 3% of other substances including minerals (Tonukari, 2004).

During the microbial production of lactic acid major impurity is the cell mass, which is separated easily from the product. The important economic factors in the industrial production of lactic acid via fermentation process includes the following (1) the optimization of the production medium, (2) high product yields, (3) productivity, and (5) the concentration of products formed, which ultimately influences the down-stream processing costs (Abdel-Rahman *et al*, 2011).

Objective

The main objectives of the current study was to select a *lactobacillus* strain which efficiently produces L-(+)-Lactic acid and to develop a cost effective medium using Cassava as the main substrate to produce high yield of L-(+)-Lactic acid, in large scale fermentation.

Methods

Processing Cassava Powder

Fresh cassava was washed with water and both outer dry and inner layers were removed. The peeled cassava was cut into small pieces and dried at 65 °C for 48 hours (hrs). The dried cassava was ground to make a powder using a motor and pestle. The acid hydrolysis of the starch containing cassava was performed using

different concentrations of HCl (0.5, 1, 1.5, 2, 2.5, 3, 5, 8 and 10 %). A weight of 1 g of cassava powder was mixed with different HCl solutions having different concentration and kept at 120 °C for 10 minutes (mins). The amount of reducing sugar produced by different concentration of acid was detected by Benedict's test (Pratt, 2011).

Selection of microorganisms for fermentation

Lactobacillus casei (ATCC No: 393) and *Lactobacillus delbrueckii* (ATCC No: 15808) were used as the bacterial strains to produce L-(+)-Lactic acid (Panesar et al, 2010 and Chang et al, 1999).

Preparation of a Pilot Scale Fermentation Medium

The lactic acid fermentation was conducted with a 100 ml of acid hydrolyzed cassava product namely; medium A, B, C, D, and E enriched with some nutrient sources (Table 1). Each medium was neutralized using 2% HCl and 2 N NaOH (pH 5.5). The media A, B, C, D and E were fermented with *L. casei* and *L. delbrueckii* separately. The fermentation was carried out in a shaking incubator for 48 hrs at 37 °C for 150 rpm.

Table 1. Composition of different media used

Medium	Composition
A	Cassava- 5 g, Yeast extract-1.5 g, K ₂ HPO ₄ -0.41 g, KH ₂ PO ₄ -0.56 g
B	Cassava- 5 g, Yeast extract 1.5g, MgSO ₄ .7H ₂ SO ₄ - 1 g, (NH ₄) ₂ SO ₄ -1 g
C	Cassava- 5 g, Yeast extract- 5g, K ₂ HPO ₄ - 2 g, NaAc- 5 g, MgSO ₄ .7H ₂ SO ₄ - 0.02 g, MnSO ₄ .4H ₂ O-0.05 g
D	Cassava- 5 g, Yeast extract- 1.0g, K ₂ HPO ₄ - 0.41g, MgSO ₄ .7H ₂ SO ₄ - 1g, (NH ₄) ₂ SO ₄ -1g
E	Cassava crumble- 5 g, Yeast extract- 1.0g, K ₂ HPO ₄ - 0.41g, MgSO ₄ .7H ₂ SO ₄ - 1g, (NH ₄) ₂ SO ₄ -1g

Detection of L-(+)-Lactic Acid Using High Performance Liquid Chromatography (HPLC)

The media were centrifuged initially at 4000 rpm for 20 mins at 4°C and supernatant was re-centrifuged at 12000 rpm for 15 mins at 4°C in order to remove impurities. The High Performance Liquid Chromatography was conducted with Ultra Violet (UV) wave length 210 nm and Refractive Index Signal (RIS). A volume of 5 µl of the supernatant was filtered and injected into the pump of HPLC. Phenomenex Rezex ROA H+ (300 x 7.8 mm, 8 µm) was used as cation exchange column and deionized water was used as the mobile phase. A temperature of 30°C was provided with a flow rate of 0.4 mL/min. The peak area corresponded to the L-(+)-lactic acid was used to quantify the amount of L-(+)-Lactic Acid. The medium which was given the high yield of L-(+)-lactic acid was identified for large scale fermentation.

Preparation of Medium for Large Scale Fermentation and Harvesting

The medium D was prepared for 14 Liters (L) consisting 200 g of acid hydrolyzed cassava, 40 g of yeast extract, 16.4 g of K₂HPO₄, 20 g of MgSO₄.7H₂SO₄ and (NH₄)₂SO₄. The large scale fermentation was performed using New Brunswick™ BioFlo® 415 SIP Fermentor for 5 continuous days. A sample was drawn in the first day prior

to the inoculation of microorganisms (negative control) and 3 samples each from day 1, 2, 3, 4 and 5 were obtained after the microbial inoculation. The fermentation was repeated for two times and quantity of the L-(+)-lactic acid produces in each day was detected using the HPLC as described above.

Results

Acid hydrolysis of cassava

The Benedict's test provided different amount of reducing sugars. Green color precipitate indicated 0.5 % of sugars, while yellow color precipitate as 1 %, orange color as 1.5% and brick red color indicated as 2 % of reducing sugar levels. The results of the Benedict's test are shown in table 2. The highest amount of reducing sugar was observed from the cassava product which hydrolyzed with 2 % HCl

Table 2 Results of the Benedict's test

Concentration of HCl added (%)	Results
0.5	+
1	+
1.5	+
2	++
2.5	++
3	++
5	-
8	-
10	-

+: Low green high yellow precipitate

++: High yellow color precipitate

-: No color change (Negative)

Table 3 Results of pilot scale fermentation of different media using 210 nm

Medium	milli Absorbance Units per second(mAU*s) by each bacterial strain	
	<i>L. casei</i>	<i>L. delbrueckii</i>
Standard	225.1	225.1
A	454.0	99.5
B	374.5	32.7
C	1683.7	738.3
D	1076.5	635.5
E	483.8	103.6

Quantification of Lactic Acid Detection by HPLC for small scale fermentation

The HPLC results of different media are given in table 3. The highest milli Absorbance Units per second (mAU*s) was observed from the C and D media fermented with *L. casei* bacterial strain. However, the medium contained some constituents in the MRS media which the ingredients are costly.

Therefore, it is not suitable to use as a low cost fermentation medium. Hence, the medium D was identified as the suitable medium. All media which fermented with *L. delbrueckii* were resulted low mAU*s values with compare to *L. casei* (Table 3). Therefore, *L. casei* indicated high L-(+)-lactic acid concentrations in the fermented products (Figure 1).

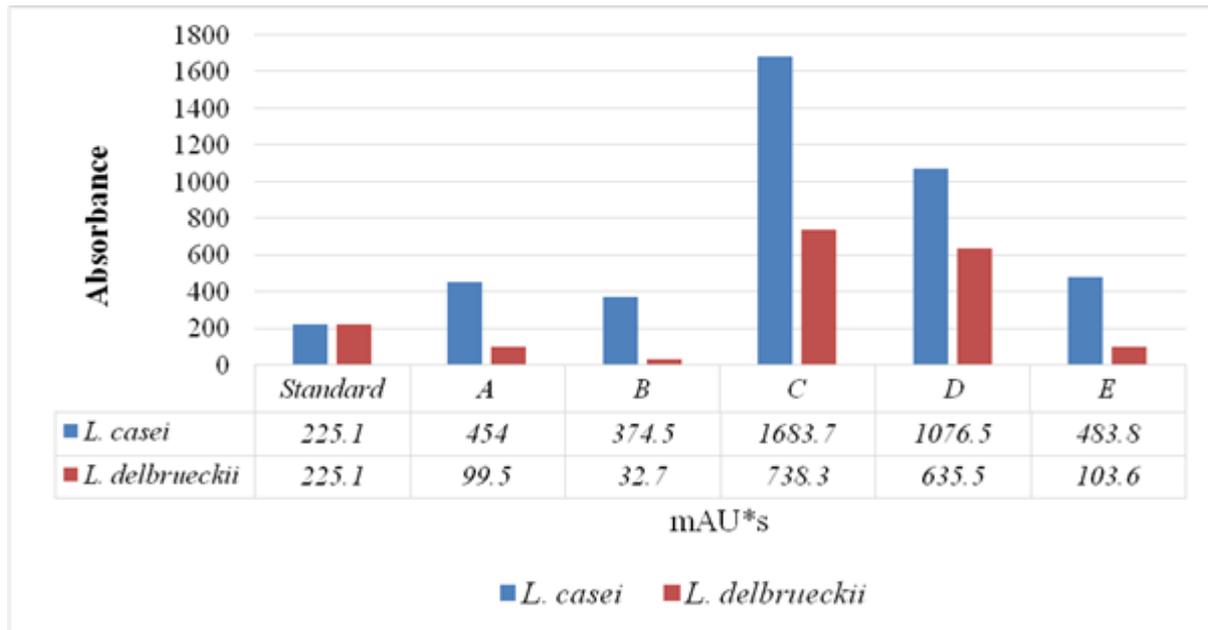


Figure 1: Productivity of L-(+)-lactic acid in five different media fermented with *L. casei* and *L. delbrueckii*

Quantification of Lactic Acid Detection by HPLC for large scale fermentation

The concentration of L-(+)-lactic acid detected from each sampling day is shown in table 4. Overall, the highest concentration of L-(+)-lactic acid was identified in the fourth day of the fermentation process.

The medium was changed in to light brown to dark brown with increasing time. It was further observed that the viscosity of the medium was also increased with respect to time. However, after the fourth day of fermentation, the quantity of L-(+)-lactic acid was gradually decreased. This may be due to conversion of lactic acid in to pyruvate by lactate dehydrogenase enzyme activity of the microorganisms.

Table 4 Results of large scale fermentation of D medium with L. casei using HPLC at 210 nm

Sampling day	milli Absorbance Units per second at 210 nm	Concentration of L- lactic acid g/L
01	604.4	0.15
02	1076.5	0.29
03	1522.4	0.43
04	8657.3	2.57
05	6526.5	1.93

Validation of Results

The concentration of the L-(+)-lactic acid was determined using a standard curve (Figure 2) using a stock solution of L-(+)-lactic acid having 1.1 mg/l concentration. The R value of the According to the standard curve, the R value was 0.99.

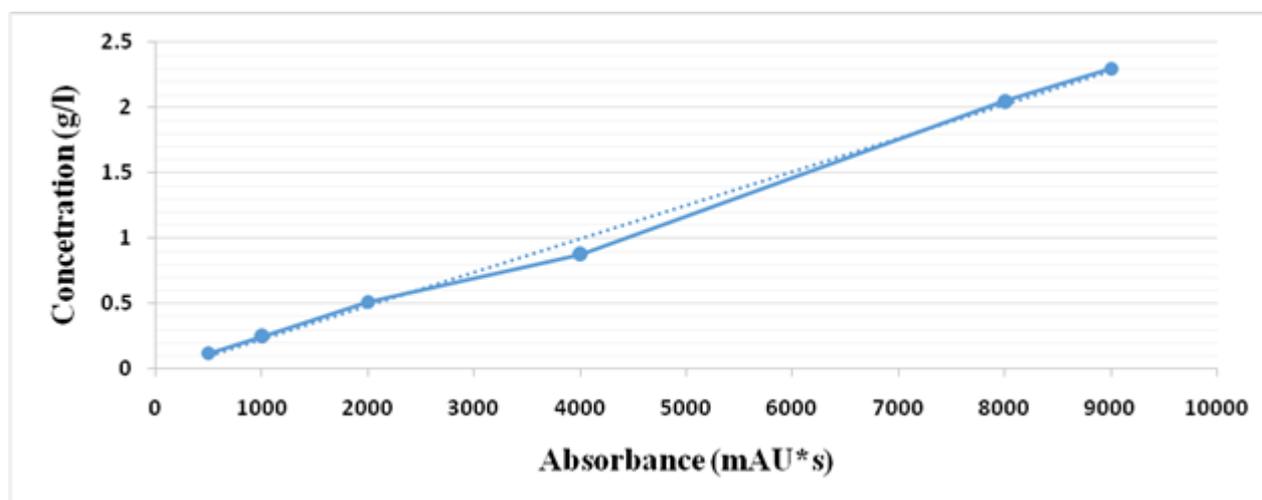


Figure 2: Standard curve

Discussion

Poly lactic acid or poly lactide (PLA) is a thermo plastic and biodegradable aliphatic polyester derived from naturally occurring organic acid (lactic acid). It is inexpensive, dimensionally stable and harder than Polytetrafluoroethylene (PTFE). It melts at lower temperature (180 - 220 °C) with glass transition temperature 60 - 65 °C. Due to their excellent biocompatibility and mechanical properties, it has been used extensively in different fields such as polymer engineering, tissue engineering drug delivery systems and various medical implants of paramount significance such as sutures in surgical applications. It composites many applications in biomedical devices from fibers to subcutaneous sutures and to regenerative surgery implants (Pawar *et al*, 2014). This has also become a promising eco-friendly biopolymer for use in the human body.

The *Lacto bacillus* genus has been generally known as the major producer strain of lactic acid. These organisms have limited ability for synthesizing their own growth factors, mainly B vitamins and amino acids. Typically, they require carbon and nitrogen source sand diverse elements in the form of carbohydrates, amino acids,

vitamins and minerals. In addition, the use of assortments of amino acids, peptides and amides, stimulate growth of Lactic Acid Bacteria (LAB) yielding much higher values than those obtained with free amino acids. LAB growth is also influenced by fatty acid and phosphate which are the most important salt in the LA fermentation (Quintero *et al*, 2013).

However, the process of microbial fermentation of lactic acid is extremely expensive procedure for a developing country. This leads to the use of other sources such as soybean, potato, wood, corn liquor, and molasses are to be used for the production of lactic acid. Among these, starchy grains like sweet sorghum, wheat, corn, cassava, potato, rice and barley, and cellulosic materials, are widely used having the advantages such as low price, availability and renewable characteristics over other sources (Farooq *et al*, 2014 and Wee *et al*, 2006).

The fermentation process also requires the nitrogenous materials for the growth of the microorganisms. This need is satisfied by utilization of whey permeate, yeast extract, malt sprouts, grass extract, peptones, beef extract, casein hydrolyte with supplementation of vitamins that have been used with the carbohydrate source for fast and heavy growth. Among these Yeast extract is found to be the most effective supplement, thus it was utilized a nitrogen source in the research as well (Quintero *et al*, 2013).

It is noticed that for many lactic acid bacteria (LAB) strains cannot serve only utilizing ammonium as the primary nitrogen source; nevertheless, some display influence on the amino acid metabolism of the LAB strain. Conversely, the amount of minerals found in commercial complex media seems to be sufficient for lactic acid metabolism. With the expectation provided, it can be specified that the cassava flour is the main fundamental constituent for the optimized media in the research. Thus, cassava has become a suitable substrate for present study, aimed to biosynthesis of lactic acid via a microbial fermentation process. According to Tonukari, 2004 (Tonukari, 2004), 81.48 % cassava flour has 99% of starch hydrolyzed, when 300 g/L of cassava flour medium was prepared, accomplishing about 76% of glucose content; the residual part is conducted with maltodextrines.

The current results suggests that the medium D containing K_2HPO_4 , $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$, with acid hydrolyzed cassava and yeast extract are more suitable as a low cost fermentation media since it does not contain costly substances. Based on this analysis, and besides reducing sugars, cassava flour has different ions including sodium, iron and magnesium, amino acids and proteins that might have favored bacterial growth and product biosynthesis (Afolabi *et al*, 2012).

Even though *Lactobacillus* is a facultative anaerobic organism, its metabolic activity is enhanced in the absence of oxygen, among other factors, by the need of the oxidized form of the cofactor NAD^+ , which is reduced during the catabolic activity. The NADH donates its extra electrons to the pyruvate molecule formed during glycolysis; since the NADH has lost electrons, NAD^+ regenerates and is again available for glycolysis. However, *Lactobacillus* as a facultative anaerobic bacterium, is capable to ferment and experience cellular respiration while oxygen is present; this process is known as hetero fermentative. Biosynthesis of lactic acid in a hetero fermentative process produces carbon dioxide and ethanol, therefore implementation of lactic acid yield is reduced (Quintero *et al*, 2013).

Lactobacillus casei species belongs to different strains, which can be isolated from various environments, and some of the *L. casei* are currently used in commercial products as probiotics. These species are subjected in to studies regarding their numerous taxonomic groups, since many strains were previously classified according to their characterization, which was similar to *L. casei*. It was further notices *L. casei* sub sp. *casei* ATCC 393^T was having genotypic, phenotypic and phylogenetic differences when compared to the standing type of *L. casei*.

The strain *L. casei* sub sp. *casei* ATCC 393^T is especially been used in studies based on the biological process of fermentation of sugars such as glucose, lactose, citrate and pyruvate, comparative studies on and molecular characterization of the enzyme L-lactate dehydrogenase, also in the characterization of an intracellular β

glucosidase, proteolytic activity and studies on the composition of the cell wall, antibiotic resistance and adherence factors (Acedo-Félix and Pe´rez-Martí´ne, 2003).

Furthermore, genus *Lactobacillus* was reported to be primarily in the studied by *L. casei* strains, especially through the sub sp ATCC 393^T; the sup sp was used to retrieve information regarding the purpose of isolation and characterization of extrachromosomal genetic elements. Moreover, between the standing type strain and the plasmid-cured strain, the plasmid-cured strains showed a higher potential for lactose metabolism on research studies. Thus, due to this reason it was observed it carried a second lactose-specific transport and hydrolysis system, which is namely the lactose phosphoenolpyruvate phosphotransferase system (PTS) and a 6-phospho-b-galactosidase respectively. (Acedo-Félix and Pe´rez-Martí´ne, 2003)

In the present study *L. casei* and *L. delbrueckii* were used since they specifically produce L-(+)-Lactic acid (Panesar *et al*, 2010 and Chang *et al*, 1999) which we are interested in the current research. *L. casei* and *L. delbrueckii* are gram-positive lactic acid bacteria. They are non-motile, have non-spore-forming rods and cocci. These lactic acid bacteria grow under anaerobic conditions, which help them not to use oxygen for their energy manufacture, but they are also capable of growing with presence of oxygen (Coeuret *et al*, 2003). The current study observed the highest efficiency from *L. casei* than *L. delbrueckii*. According to the literature, *L. delbrueckii* requires higher nutrient level to grow (Coeuret *et al*, 2003). Hence, large scale fermentation will not be economical.

During the lactic acid production in the present study, it was noticed the microbial fermentation process was inhibited after the 4th day of inoculation of microorganisms. Further reading on this matter, it was understood this process conventionally suffer from end-product inhibition. This is due to the reason an un-dissociated lactic acid passes through the membrane of bacteria and dissociates inside the bacterial cell. Thus it concluded the mechanism of inhibition of lactic acid is related to the solubility of the un-dissociated lactic acid which is present in the cytoplasmic membrane of the bacteria and the insolubility of dissociated lactate, which produces acidification of cytoplasm, thus the proton motive forces is failed. This phenomena eventually inducements the transmembrane pH gradient and the amount of energy available for cell growth is decreased. Thus, to lighten the inhibitory effect of lactic acid during the fermentation process via microbes, it is endorsed to remove selectively in situ from the fermentation broth or media (Wee *et al*, 2006).

The lactate dehydrogenase enzyme in the microorganisms are not only produces lactic acid from simple sugars but also converts the lactic acid into pyruvate. Thus, the amount of lactic acid is easily converted to pyruvate. This pyruvate, and sugar content, which are not utilized by bacteria form disturbance during detection of lactic acid via HPLC; forming different peaks. The peak area of the standard lactic acid solution is much lesser than the lactic acid produced from the media, this is due to the reason as the standard lactic acid solution is diluted than the lactic acid found in the media.

The fermentation method used in the current study is the batch method. Other commonly used methods for fermentation process for lactic acid production includes fed-batch, repeated batch, and continuous fermentations. It was also been noticed from previous studies a higher concentrations of lactic acid could be obtained in batch and fed-batch cultures than in continuous cultures, whereas by the use of continuous cultures higher productivity could be achieved. Another added advantage of the continuous culture compared to the batch culture, is the possibility to continue the process for a longer period of time (Wee *et al*, 2006).

The allosteric enzyme L- lactate dehydrogenase in *L. casei* is with fructose 1, 6- bisphosphate (FDP) and the Mn²⁺ acts as the cofactor in some cases. The LDH in *L. casei* found in eukaryotes and in *L. casei* living in vertebrates express a 37 % and 76 % similarity respectively, but 70 % and 86 % similarity respectively in their active sites of LDH. This phenomena explains us that the essential parts of LDH enzyme has been conserved. In comparison to the vertebrate LDH in *L. casei* is found to lack 12-amino acid residues at the N- terminus, which is found to be a common characteristic of bacterial enzymes irrespective of the allosteric behavior. *L. casei* also

carries a C end of the protein with 7 additional amino acid residues, however it is not identified whether this characteristic of L- lactate dehydrogenase enzymes of bacteria as no complete sequence of other bacterial enzymes available (Narayanan et al, 2014).

Furthermore, after the production of lactic acid in the large scale, the medium D, it would be purified and polymerized to obtain the PLA biosynthetic polymer to be used as orthopedic devices. L-PLA can have two crystalline modifications, α and β conformation. The synthesise of high-molecular-weight PGA and PLA is constructed by opening the polymerized ring of the cyclic lactide. Catalysts used in the polymerization process include antimony, zinc, or lead. However, low-molecular-weight homo- and co-polyesters of lactic acid will also be synthesized by direct poly-condensation in the presence of water without using catalysts.

As mentioned earlier, the polymers PLA and PGA are biodegrade mainly by hydrolytic scission which occurs nonspecifically. By the simple hydrolysis, the polymer chains in the ester linkages are fundamentally cleaved. PLA undergoes hydrolytic scission to its monomeric form, thus lactic acid which is eliminated from the body is integrated into the tricarboxylic acid cycle. The eliminated lactic acid is finally excreted by its principal elimination path as respiration via the lungs as CO₂ and in urine. In a study done on rats, the PLA implants were labeled with radioactive carbon and placed internally in rats for 3 months, as a result there was no significant radio activity found in the urine or feces, which confirmed that the polymer is degraded and probably eliminated through CO₂ during respiration. Further, PGA can be broken down in two ways, by hydrolysis sand by nonspecific esterase and carboxypeptidases (Agrawal *et al*, 1995).

Conclusion

According to the study performed *L. casei* could be selected as the efficient and low cost LAB strain for the production of L-(+)-lactic acid in industrial scale fermentation. Further, Medium D was identified as the effective low cost medium for large scale fermentation for the production of lactic acid with *L. casei*. Purification and polymerization need to be performed in order to synthesis the biopolymer. It is suggested that *L. casei* and medium D together would be effective in producing a high yield of L-(+)-Lactic acid which could produce biodegradable polymers for orthopedic devices.

References

- Afolabi, A. S., Mobee, O. S., and Abdulkareem, A. S. 2012, Production of Lactic Acid from Cassava Starch Hydrolysis using Immobilized *Lactobacillus Casei* in a Fibrous Bed Bioreactor. Proceedings of the World Congress on Engineering, 3, (London, U.K.), Date of access: 15/ 8/2015. http://www.iaeng.org/publication/WCE2012/WCE2012_pp1570-1573.pdf.
- Agrawal, C. M., Niederauer, G. G., and Athanasiou, K.A, 1995, Fabrication and characterization of PLA-PGA orthopedic implants. Tissue Engineering, 1(3), 241-252.
- Chang, D. E., Jung, H. C., Rhee, J. S, and Pan, J. G, 1999, Homofermentative Production of D- or L-Lactate in Metabolically Engineered Escherichia coli RR1. Applied and Environmental Microbiology, 65(4), 1384-1389.
- Coeuret, V., Dubernet, S., Bernardeau, M, Gueguen, M., and Vernoux, J., 2003, Isolation, characterization and identification of lactobacilli focusing mainly on cheeses and other dairy products. EDP Science, 83(4), 269-306, 2003.Date of access: 10/10/2015. <https://hal.archives-ouvertes.fr/hal-00895504/document>.
- Farooq, M., Anjum, M., Zahoor, M., Rahman, S., Randhawa, W., Ahmed, M., and Akram, M., 2014, Optimization of lactic acid production from cheap raw material: Sugarcane molasses. Pakistan Journal of Botany, 44(1), pp. 333-338.
- Ghaffar, T., Irshad, M., Anwar, Z., Aqil, T., Zulifqar, Z., Tariq, A., Kamran, M., Ehsan, N., and Mehmood, S., 2014, Recent trends in Lactic Acid biotechnology: A brief review on production to purification. Radiation Research and Applied Sciences, 7, 222-229.

- Kaihara, S., Matsumura, S., Mikos, A.G., and Fisher, J.P., 2007, Synthesis of Poly (L-lactide) and Polyglycolide by ring- opening polymerization. *Nature Protocol*, 2(11), 2767-2771.
- Middleton, J.C., and Tiptonj. A., 2000, Synthetic Biodegradable Polymers as Orthopedic Devices. *Biomaterials*, 21, 2335-2346.
- Narayanan, N., Roychoudhury, P., and Srivastava, A., 2013, L (+) lactic acid fermentation and its product polymerization. *Electronic Journal of Biotechnology*, 7(2), 167-179.
- Panesar, P.S., Kennedy, J.F., Knill, C.J., and Kosseva, M., 2010, Production of L (+) Lactic Acid using *Lactobacillus casei* from Whey. *Brazilian Archives of Biology and Technology*, 53(1), 219-226.
- Pawar, R.P., Tekale, S.U., Shisodia, S.U., Totre, J.T., and Domb, A.J, 2014, Biomedical Applications of Poly (Lactic Acid). *Recent Patents on Regenerative Medicine*, 4, 40-51.
- Pratt, W. C., 2011, A Biology Laboratory Exercise Using Macromolecule Assays to Distinguish Four Types of Milk. *Journal of Microbiology and Biology Education*, 12(1), 40-51.
- Quintero, M., Acosta, C., Mejia,G., Rios, E., and Torres, L., 2014, Lactic acid production via Cassava-flourhydrolysate Fermentation. *Vitae Journals*, 19(3), 287-293.
- Taskila, S., and Ojamo, H., 2013, The Current Status and Future Expectations in Industrial Production of Lactic Acid by Lactic Acid Bacteria. *InTech Journals*, Date of access: 13/11/2015 <http://dx.doi.org/10.5772/51282>.
- Tonukari, N. J., 2004, Cassava and the future of starch. *Electronic Journal of Biotechnology*, 7 (1), 5.
- Wee, Y. J., Kim, J.N., and Ryu, H.W., 2006, Biotechnological production of lactic acid and its recent applications. *Food Technology and Biotechnology*, 4(2), 163-172.
- Nampoothiri, K. M., Nair, N. R., and John, R. P., 2010, An overview of the recent developments in polylactide (PLA) research. *BioresourceTechnology* ; 101 8493-8501
- Abdel-Rahman. M. A., Tashiro. Y., Sonomoto. K., 2011, Lactic acid production from lignocellulose-derived sugars using lactic acidbacteria: Overview and limits. *Journal of Biotechnology*, 15(6), 286-301.
- Jarvi's, L., 2001, Lactic acid outlook up as poly lactide nears market. *Chemical Market Reporter*. Feb. 26, 14.
- Zaunmuller, T., Eichert, M., Richter, H., and Unden, G., 2006, Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. *Applied Microbiology and Biotechnology*; 72 421-429.
- Fakhravar, S., Najafpour, G., Zeinali-Heris, S., Izadi, M., and Fakhravar, M., 2012, Fermentative Lactic Acid from Deproteinized Whey Using *Lactobacillus bulgaricus* Batch Culture, *World Applied Sciences Journal* 17 (9): 1083-1086
- Department Of Agriculture. 2006, Cassava: *Manihotesculenta*, Governmnet of Sri Lanka. Viewed 5 May 2015. Retrieved from <http://www.agridept.gov.lk/index.php/en/crop-recommendations/1456>
- Acedo-Fe Lix, E., and Pe Rez-Marti Ne, G., 2003, Significant differences between *Lactobacillus casei* subsp. Casei ATCC 393^T and a commonly used plasmid-cured derivative revealed by a polyphasic study. *International Journal of Systematic and Evolutionary Microbiology*, 53(1), 67