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Lactic Acid Fermentation and Production of Polylactic Acid From Domestic Wastes Using *Lactobacillus Delbruekii* Subsp. *LaCTIS*

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ABSTRACT
Biodegradable plastics from wastes enables both recycling of the waste and also producing a value added product from it. The production of plastics, and articles produced from them, is expanding systematically. Simultaneously, the amount of waste is increasing because the majority of conventional plastics are resistant to the long-lasting action of weather and/or drastic biological conditions. Both recycling and combustion are processes which permit only a partial solution of the above-mentioned problem. Thus, in recent years we have observed intensive development in investigations into biodegradable polymers. It seems that one polymer which may meet our requirements and replace the majority of popular plastics on the market is polylactic acid (PLA). Synthetic and natural biopolymers are finding their way into a variety of applications in materials science and biointerface engineering, such as tissue engineering scaffolds, drug delivery matrices, and as detectors and transducers in biosensors.Lactic acid fermentation is carried out using Lactobacillus delbruekii subsp. lactis MTCC 911 in various domestic wastes such as fruit waste, vegetable waste, and whey waste, followed by the purification of lactic acid by butyl esterification from the fermentation broth, and polymerization of the lactic acid by polycondensation into polylactic acid, the biodegradable plastic. Before and after fermentation, total sugar present in various substrate used is calculated by phenol sulphuric acid method of sugar analysis. Total substrate consumed and substrate conversion is calculated further for each fermentation medium and compared to standard MRS medium. Among the wastes, Whey waste shows high substrate consumption of 17.25g/l and minimal substrate conversion of 14.20%. The results show that Whey waste is the most suitable medium for Bioplastic production.

KEYWORDS
Lactic acid fermentation, Bioplastic, Polylactic acid, Polycondensation, Esterification, Whey waste

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INTRODUCTION

Biodegradable polymers are natural or synthetic in origin and are degraded in vivo, either hydrolytically or enzymatically to produce biocompatible, toxicologically safe by-products that are further eliminated by the normal metabolic pathways [1]. Biodegradable polymers are degraded in biological environments, where living cells or microorganism are present, such as soil, composts, seas, rivers, lakes, bodies of human beings and animals through enzymatic or non-enzymatic hydrolysis [2].

In recent years, much concern has increased on the deterioration of our environment due to solid waste pollution. One way to solve that problem is replacing commodity synthetic polymers with biodegradable polymers. Among them, aliphatic polyester is one of the most promising biodegradable materials because they are readily susceptible to biological attack [3]. PLA, a biodegradable aliphatic polyester, produced from renewable resources has received much attention in the research of alternative biodegradable polymers [4]. Lactides and lactic acid monomers are obtained from the fermentation of crop like corn starch and sugar feed stocks [5]. Polymerization of lactic acid into PLA produces a biodegradable thermoplastic polyester with good biocompatibility and physical properties, such as high mechanical strength, thermoplasticity and fabricability [6].

Disposal of PLA hysterics with exiting systems including the additional option of composting. Long term, with correct infra structure, PLA products could be recycled back to a monomer and into polymers. This eco-friendly plastics make our earth clean and green [7].

Conventional synthetic polymers rely on reserves of oil and gas for their monomer source and energy to manufacture. These reserves of fossil fuel take millions of years to regenerate and are a declining resource. In contrast, the monomer used to manufacture polylactic acid is obtained from annually renewable crops. Energy from the sun promotes photosynthesis within the plant cells; carbon dioxide and water from the atmosphere are converted into starch. This starch is readily extracted from plant matter and converted to a fermentable sugar (e.g. glucose) by enzymatic hydrolysis. The carbon and other elements in these natural sugars are then converted to lactic acid through fermentation [8].

Organic wastes from domestic garbage are high in moisture content. The waste generated from kitchen and food-processing industries are rich in carbon content. The municipal solid wastes are usually incinerated or land filled but these processes generate high environmental impacts. Since landfill space is limited, uncontrolled fermentation of organic waste in landfill causes secondary problems such as methane emissions, global warming and air pollution. So an environmentally friendly mode of treating municipal waste is required.

Treatment of domestic organic waste using microbiological process improves the effective usage of waste and to develop value added products from the wastes. There is an alternate method to reuse food wastes such as direct composting and methane fermentation, which produces fertilizers and biogas, but these processes have been applied only in some areas. On the other hand lactic acid fermentation using lactic acid bacteria with the organic wastes as substrate is an effective solution [9].

The proven degradability in biological systems, biocompatibility and the possibility of tailoring the properties to a wide range have made lactic acid derivatives well suited for a range of applications. The environmental issues that have gained importance during the last decade have resulted in efforts to applying the lactic acid polymers for medical applications and as packaging materials. Lactic acid can be derived from a wide range of renewable materials and can easily fit into municipal waste management systems [10].

Kious [11] reported about Lactobacillus and production of lactic acid. Two strains of lactobacillus were used to optimize the production of lactic acid. The results are promising for higher yield of lactic acid to be produced using lactobacillus especially future investigation into fermentation conducted under pH control.

Study on sugar utilization by Audet *et al.* [12] reported that sugar utilization by free and entrapped cells of Streptococcus salivarius subsp. thermophilus, Lactobacillus delbruekii subsp. bulgaricus and Lactobacillus lactis subsp. lactis in a whey permeate medium.Immobilization affects the fermentation rate of lactic acid bacteria, especially Lactobacillus delbruekii subsp. bulgaricus. Entrapped cells of L.delbruekii subsp. bulgaricus demonstrated a lower lactic acid production than did free cells in batch fermentation

Ilmen *et al.* [13] suggested about the efficient production of L-Lactic acid from xylose by Pichia stipitis. Pichia stipitis, a yeast that naturally ferment xylose was genetically engineered for L-(+)-lactate production. The results showed that for the first time lactate production from xylose by a yeast species is efficient and feasible. This is encouraging for further development of yeast-based bioprocesses to produce lactate from lignocellulosic raw materials.

Lactic acid has been produced commercially by fermentation since 1883. The major application of lactic acid is in the food industry as an additive and preservative. Other applications include use as a pharmaceutical intermediate, lactate ester, which is an alternative solvent to glycol ether. Lactic acid-derived polymers are becoming increasingly important because of their application within drug delivery systems and their biodegradable and thermoplastic nature means that they can be produced as high volume biodegradable plastics for packaging and other applications. However, this potential can only be realised if the cost of production is competitive on a global scale [14].

MATERIALS AND METHODS

PRODUCTION OF LACTIC ACID

Inoculum Preparation

Lactobacillus delbruekii subsp. lactis MTCC 911 is purchased from MTCC (Microbial Type Culture Collection, Chandigarh, India).The culture is inoculated in the MRS medium and incubated at pH of about 6.5 for 24 hours at 37°C. This acts as the seed culture for inoculating in the sterilized wastes medium.

Fermentation In Various Wastes

Fruit wastes, vegetable wastes and whey were collected from household. One kg of each waste is grounded along with 1100 ml of distilled water and filtered to obtain the filtrate which is used as the fermentation medium. All the above wastes will have different chemical compositions of carbohydrates, lignin, cellulose, hemi cellulose, protein and lipid.In 1000 ml of the sterilized wastes medium, 1% (10 ml) of 24 hours bacterial innoculum was added and incubated at pH of about 6.5 for 24 hrs at 37°C.

PURIFICATION OF LACTIC ACID: The fermented broth was allowed for centrifugation for about 10,000rpm for 20 minutes. The pellets were discarded and the supernatant was concentrated using a distillation column at 100°C. The distillate was placed in a distillation column. After n-butanol was added and the distillate was heated in an oil bath at 150-160°C and rotated periodically. The azeotropic vapour of water and n-butanol was collected by a condenser connected to the top of the distillation column and separated into water and n-butanol phases in a separator.

The n-butanol, as the upper phase, was refluxed to the top of the column, where as the lower phase was extracted. The reaction mixture was filtered to remove solid precipitates and the filtrate (butyl lactate) was evaporated using a distillation column at 200°C.

The butyl lactate purified by distillation was placed within the hydrolyzing vessel and heated between 95-110°C. The water phase was refluxed to the top of the column and the n-butanol phase was separated from lactic acid in the vessel [15].

POLYMERIZATION OF LACTIC ACID

To 100g of lactic acid produced was dissolved in 375 ml of p-xylene in a three necked flask. 1g of stannous chloride was used as a catalyst to the solution and the reaction mixture was heated. The water was azeotropically distilled off and fresh solvent was added during the distillation to keep the reagent concentration constant. The reaction was carried out until a clear distillate appeared in the receiver. Then a tube packed with 40-50g of 4A dry molecular sieves was mounted onto the apparatus and the reaction was continued at 138°C for several hours in a closed circulation system for the solvent. During the synthesis the control samples were taken out, until the reaction mixture attained the maximum viscosity.

The resulting solution was poured into an excess of methanol (of five times greater volume). The resultant white powder was washed several times with methanol and dried at 80°C for 2 hours. The PLA/starch blends were prepared in the ratio of 30:70. The polymeric solution was heated at 120°C for 3 hours. The solution was moulded into thin sheets and dried at 80°C [16].

PHENOL SULPHURIC ACID METHOD FOR SUGAR ANALYSIS

The total sugar concentration was estimated spectrophotometrically by phenol sulphuric acid method.Initial total sugar concentration (S_0), Final total sugar concentration (S), Substrate consumed (S_0-S), Substrate conversion (SC) were calculated following Cock et al., 2007.

RESULTS AND DISCUSSION

Lactobacillus delbruekii subsp. *lactis* MTCC 911 was inoculated in various domestic wastes and MRS medium and incubated for 24 hours at 37°C.The centrifuged and concentrated broth was esterified with n-butanol (150°C-160°C) and distilled in the form of butyl lactate (130°C, 98% yield) which was then hydrolyzed between 95-110°C. soluble proteins and salts were precipitated with n-butanol. Esters, such as those of acetic acid and propionic acid, were separated during this part of the reaction. Condensed water and n-butanol were recycled for subsequent esterification. This step consumed relatively high amount of energy but yielded lactic acid of very high purity. Among the wastes lactic acid production was high in whey of about 6.75 g/l and in the MRS medium of about 52.2g/l (Fig. 1).Synthesis of PLA using p-xylene was carried out in the presence of stannous chloride as catalyst at 138°C. PLA/Starch were blended and moulded into sheets. Before and after incubation, total sugar analysis of the medium was determined using phenol sulphuric acid method (Fig. 2). From the initial and final total sugar concentration, substrate consumed by *Lactobacillus delbruekii* subsp. *lactis* MTCC 911 in various domestic wastes is determined followed by the determination of substrate conversion. It was found that among wastes whey shows high substrate consumption of 17.25 g/l and minimal substrate conversion of 14.20% (Fig. 3).

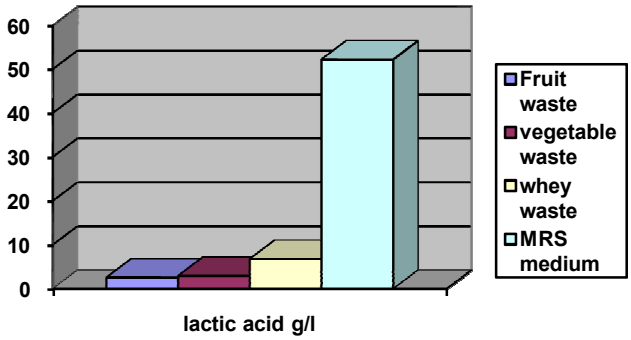

Fig. 1: Production of Lactic acid by *Lactobacillus Delbruekii* In Various Domestic Wastes

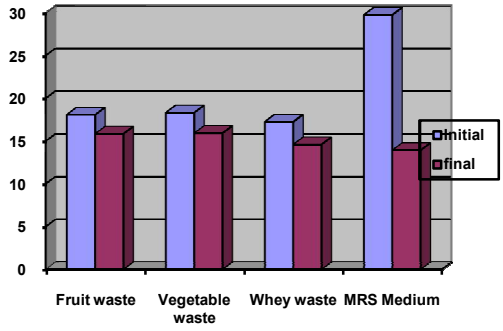

Fig. 2 : Total Sugar Concentration In VariousDomestic Wastes

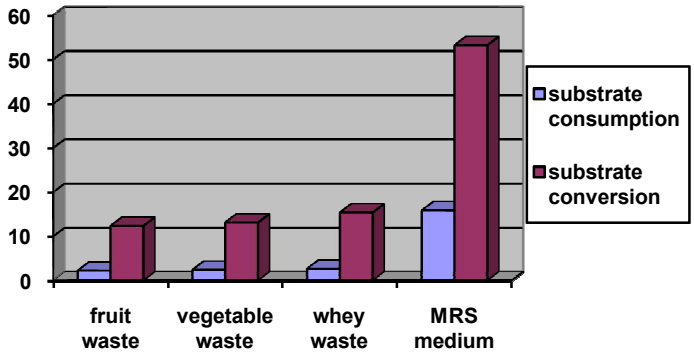

Fig. 3 : Substrate Utilization By *Lactobacillus Delbruekii* In Various Domestic Wastes

CONCLUSION

The degradability in biological systems, biocompatibility and the possibility of tailoring the properties to a wide range have made lactic acid derivatives well suited for a range of applications. The environmental issues that have gained importance during the last decade have resulted in efforts to applying the lactic acid polymers for medical applications and as packaging materials.In the international market, natural form of polymers is preferred to be used for medical purposes compared to that produced by chemical or enzymatic process. Lactic acid can be derived from a wide range of renewable materials and can easily fit into municipal waste management systems. Several major agriculture processing and chemical industries have identified and built lactic acid plants and has plans for major large-scale plants in future. Several novel processes are being deployed for facile production and separation of lactic acid and their manufacturing costs and economics have attractive potential in large scale operations.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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