

Pivotal role of microbes in solid waste management

Abstract

Lactic acid bacteria (LAB) are used widely in food microbiology as probiotics and biopreservatives to extend shelf life of food items. Lactic acid produced by LAB strains are precursor of polylactic acid (PLA) that has growing demand as bioplastics that are biodegradable in nature replacing traditional plastics. LAB have the potential to utilize polysaccharides from various sources and produce lactic acid. The aim of this study is to identify lactic acid producing strains of lactic acid bacteria (LAB) that have efficiency in utilizing biodegradable fragment of domestic solid waste collected from residential areas of Coimbatore municipality for three days. The main objective of this study is to convert the biodegradable solid waste into lactic acid, a precursor of Poly lactic acid-PLA used as bioplastic. Important chemical parameters of the solid waste like phosphate estimation, nitrate estimation, Total organic carbon estimation were done following Indian standard analytical methods. Glucose consumption efficiency of *Lacto bacillus sp.* in solid waste substrate was analyzed in UV spectrometer at 620nm by Anthrone method. Estimation of lactic acid produced was done in UV spectrometric analysis at 390nm using iron lactate formation. In this study, lactic acid bacteria were isolated from samples of milk, curd, idli batter and screened for lactic acid production. Three isolates were chosen for the study each one from milk, curd, idli batter and from biochemical tests and morphology confirmed as *Lacto bacillus sp.* Solid waste collected was pretreated with dilute acid heating and hydrolysate obtained was used as substrate for lactic acid production under optimized parameters. Three different substrates were chosen for lactic acid production and the results were compared. The three isolates of *Lacto bacillus sp.* were good producers of lactic acid and utilized biodegradable solid waste in an effective manner. 3-5 gm/lit of lactic acid was produced by the three strains of *Lacto bacillus sp.* Segregation of domestic solid waste collected, comprised of 40% biodegradable fragment, 40% nondegradable plastic, 20% miscellaneous waste. Lactic acid production from biodegradable portion is a preliminary step experimented in this study progressing in polylactic acid PLA pathway indirectly reducing plastic pollution. In a similar manner, nonbiodegradable plastic waste can be minimized using Exopolysaccharide pathway by *Xanthomonas sp.* that is reviewed in this study. Disposal of solid waste in an economical and greener way is a smart method of saving environment. Bioconversion of plastic into pseudoplastic xanthan gum by *Xanthomonas sp.* (through exopolysaccharide pathway) are greener techniques to crush down the accumulated plastic mountains to ground level.

Keywords

Lactic acid production, bioplastic PLA, solid waste management, pseudoplastic, bioconversion.

Introduction:

Lactic acid bacteria are utilized in the commercial production of lactic acid that has wide applications like solvent for organic and inorganic compounds; in tanning leather and dyeing wool; as a flavoring agent and preservative in processed cheese, salad dressings, pickles, and carbonated beverages; and as a raw material or a catalyst in numerous chemical processes. Ethyl lactate an ester of lactic acid is called as green solvent. PLA produced by polymerization of lactic acid is treated as bio plastics nowadays. Lactic acid bacteria (LAB) are one of the most important bacterial groups in the food industry. People all over the world have long consumed them in dairy products, and the majority are classified as "generally recognised as safe" (GRAS) microorganisms because they are nonpathogenic, suitable for technological and industrial

processes, acid and bile tolerant, and can produce antimicrobial substances. *Lactobacillus casei*, *Lactobacillus plantarum* (which produced plantaricin EF), *Lactobacillus fermentum*, and *Lactobacillus paracasei* were identified as the four most promising LAB strains with probiotic potential[1]. Lactic acid bacteria (LAB) are a diverse collection of bacteria that are used to ferment foods traditionally. Even from historic periods wine brewing from grapes in barrels, making of cheese, baking bread were in common practice. Some common lactic acid bacteria (LAB) genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Oenococcus*, *Treragenococcus*, *Vagococcus* and *Weissella*. The industrialization of food transformations has increased the economical importance of LAB. Some common fermented food items are yoghurt, cheese, curd, buttermilk, kefir, kimbucha, zabadi, koumiss, dahi, idli, dosa, appam, bread, wine, beer, vinegar, soy sauce, borhani, sauerkraut[2].

Polylactic acid (PLA) is a compostable bioplastic made by polymerizing lactic acid monomers derived from starch fermentation of feedstock by lactic acid bacteria. PLA is used as a replacement for traditional petrochemical-based plastics, primarily in food packaging containers and films, in electronics and in the production of synthetic fibers. Biocompatibility of PLA has been proven to be valuable and applicable in wound healing, tissue engineering, and biomaterial creation. To enhance the texture of PLA various surface alteration approaches like thermal, chemical, physical, plasma, and radiation have been used. Dissolving PLA in chloroform and mixing it with octadecylamine-functionalized nanodiamond (ND-ODA) to improve its mechanical properties[3]. The Technological University of Denmark carried out a study in PLA packages for yogurt, butter, margarine and cheeses and observed that it possess a very good mechanical protection, moisture barrier, protection to light, fats and gases. In addition, the process of biodegradation presents the migration of lactic acid to the products in a very little percentage, proving that the migration was classified as null and it was concluded that it is ideal for the packaging of foods with high breathing or short life, bakery, fruits and vegetables[4]. The greenhouse gas emissions caused by PLA production are negligible as the CO₂ emissions from the biodegradation of PLA are compensated by the CO₂ uptake from the environment during the growth of agricultural feedstock. PLA emits around 1600 kg of CO₂ per metric ton, compared to 1850, 2740, 4140, and 7150 kg per meter ton for polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and nylon, respectively. Compared to PET, PLA produces less smoke, has a lower specific gravity, and melts at a lower temperature. Hence replacing traditional plastics by PLA based bioplastics are ecofriendly[5].

Table 1 Use of Polylactic acid PLA as a Packaging material

PLA as a Packaging material	Literature
Food and beverage containers, cups, overwrap, blister packages, coated paper and board	[6];[7]
Yoghurt cups	[8]
Production of lunch boxes and fresh food packaging	[9]
Containers for packaging of bottled water, bottled juices and yogurts	[10]
Fully renewable and degradable packaging materials	[11]
Fully renewable and degradable packaging	[12]

materials	
Fully renewable and degradable packaging materials	[13]
Commercial thermoplastics such as PET	[14]
Food packaging including direct contact applications	[15]
Antimicrobial PLA releasing chitosan containing natamycin onto the surface of solid foods such as cheese, fruits, vegetables, meat, and poultry	[16];[17]
PLA based antimicrobial bioplastics	[18]; [19]
Compost bags, disposable bags	[20]
Biodegradable film blends of chitosan with poly(lactic acid) (PLA)	[21]
LA biopolymers as biomedicine stunts	[22]

History of fermented food products:

Fermentation processes are the oldest biotechnological techniques used in food production, and they are still among the most common processes used in the food industry today. Fermented foods, such as bread, cheese, soy sauce, wine, beer, vinegar, and many others, have been part of the human diet since the dawn of civilization[23]. Idli is a common flour-based fermented food eaten in Sri Lanka and parts of India that is prepared with lactic acid bacteria that has probiotic properties. During the fermentation period of 0 to 32 hours, the pH value steadily decreased from 6.28 to 3.72 due to production of organic acids. All of the isolates were Gram-positive, nonmotile, spore-free, and catalase negative. The biochemical and sensory properties of idli batter were altered by fermentation. The flavor, texture, and nutritional value of fermented foods are increased[24]. Lactic acid bacteria (LAB) are important in traditional cheese making, either as starter cultures that cause rapid acidification of milk or as secondary microbiota that play a role in cheese ripening. Lactic acid bacteria (LAB) are economically significant because they play an important role in the fermentation process of traditional cheeses. The microbiota of raw milk cheeses is quite complex, with numerous strains of non-starter lactic acid bacteria (NSLAB) that are essential for cheese ripening and flavor development caused through milk coagulation to maturation[25]. Kefir is a popular traditional fermented dairy product with a complex probiotic and nutritional composition. Kefir grain contains casein and other milk solids, as well as the yeasts and lactobacilli that cause the distinctive kefir fermentation and act as a starter to initiate this fermentation when introduced into fresh milk. Through various biological mechanisms, kefir-derived LAB has beneficial effects on colorectal cancer, cardiovascular disease, type 2 diabetes mellitus, obesity, kidney disease, immune system modulation, and intestinal microbiota[26]. Assessment of the beneficial and safety properties of food-associated microbes is inevitable since they engage in direct interactions with their host via the digestive system. The ability to inhibit a wide range of pathogens and antibiotic resistance suggests that beverages like borhani which carry such beneficial lactic acid bacteria *Weissella confuse* strain LAB-11 can be of particular benefits to the consumers exerting preventive effects on associated diseases. In this study, we have analyzed the pathogen inhibitory activity and antibiotic susceptibility pattern of a newly isolated lactic acid bacterium obtained from the traditional beverage borhani[27]. List of few fermented products are: Acidified milk, buttermilk, filmjolk, langfil, yoghurt, dahi, Bulgarian buttermilk, Chilka curd, zabadi ,alcoholic milk (Acidophilus yeast milk, Koumiss, kefir and Moldy milk (Villi)[28]. The

pomegranate peel waste is high in polyphenols, which are natural antioxidants and biopreservatives. The goal of this study was to create a new fermented milk beverage containing polyphenols extracted from pomegranate peel and probiotic LAB[29].

D-galactose, D-mannitol, L-rhamnose, D-glucuronic acid, and L-fucose have been classified as seaweed sugars or —non-fermentable sugars. Different species of *Lactobacillus* possess the ability to metabolize different kinds of sugar. Different strains of *Lactobacillus* will use different sugars preferentially in the production of L-lactic acid. In this study, lactic acid fermentation of 11 kinds of sugars and sugar acids by 7 *Lactobacillus* species was carried out to identify the patterns of sugar utilization and acid production[30]. This research deals with the study and development of the fermentation processes of various waste biomasses from the agro-food industries, including milk whey (MW), ricotta cheese whey (RCW), pear processing residues (PPR), potato pomace (PP), tomato pomace (PT), in order to obtain the production of LA. *Lactobacillus casei* DSM 20011 (ATCC 393), a homofermentative L(+)-LA producing bacterium has been used, starting from small-scale tests to verify of the microorganism to grow in complex medium with different carbon sources . Yields from $27.0 \pm 0.3\%$ to $46.0 \pm 0.7\%$ have been obtained[31].

1.0 Lacto bacillus sp. as solid waste degraders

Methodology involves Isolation and screening for lactic acid bacteria from milk, curd, idli batter samples. Serial dilution, spread plating, streak plating in **deMan Rogosa Sharpe** MRS agar were done to obtain pure culture of lactic acid bacteria. Biochemical tests like gram staining, catalase, oxidase, acid fermentation was done.



Fig 1. Acid Fermentation Yellow Color by LAB

Table.2 Morphological Identification of the Colonies:

Description	milk(m)	curd-c	Idli batter(I)
Size of the colony(mm)	0.5-1mm	0.5-1mm	0.5-1mm
Size appearance	medium	large	small
Form of the colony	circular	circular	circular
Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Color	White	White	White
Gram staining color	Purple	Purple	Purple
Gram staining	positive	positive	positive

According to Bergey's manual of systematic bacteriology, from the results obtained, isolates from milk(m), curd©, idli batter(I) were confirmed as *Lactobacillus sp.*

Table.3 Comparison in Bergey's manual of systematic bacteriology for lactic acid bacteria

Description	Milk(m)	Curd-c	Idli Batter(I)
Shape	rod shaped	rod shaped	rod shaped
Gram staining	positive	positive	positive
Catalase	negative	negative	negative
Oxidase test	negative	negative	negative
Acid fermentation	positive	positive	positive
Gas bubble formation	negative	negative	negative



Fig 2 . Lactic Acid Bacteria from Milk, Curd, Idli Batter in deMan Rogosa Sharpe(MRS) Agar

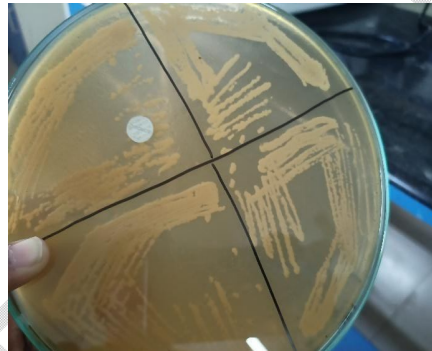


Fig-3 Streak plating of *lactobacillus* sp.

1.1 Substrate pretreatment:

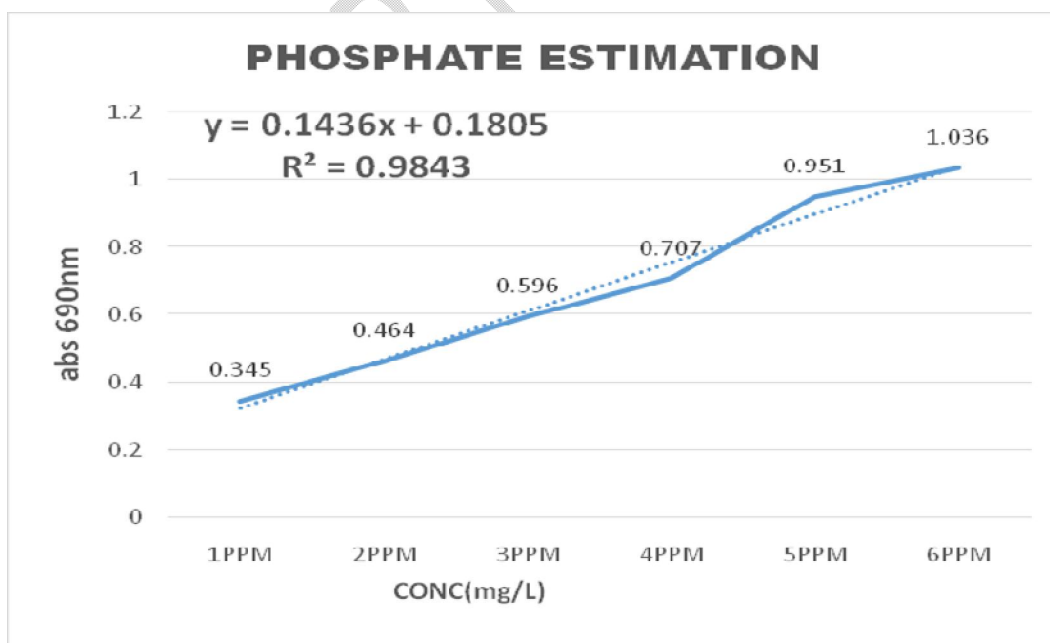
Solid waste was collected for three days in Residential area of Rathinapuri, Coimbatore weighing approximately 5kg-8kg/day from ten households. The ten samples of solid waste collected per day were mixed together and segregated as biodegradable, nondegradable plastic and miscellaneous waste categories. The process was repeated for three consecutive days. At the end of three days, three samples of solid waste were ready after processing in laboratory. The biodegradable fragment was processed in hot air oven at 80°C till the moisture was evaporated. Then the dried matter was grinded well and sieved to obtain fine particle. Solid waste hydrolysate was prepared by treating 5gm accurately weighed solid waste powder(optimum solid waste dosage) with 100ml of 0.1N sulphuric acid(5%). The

mixture was heated at 70°C for 20 minutes in hot plate in open beaker. The mixture was allowed to cool to room temperature and centrifuged at 5000rpm -15 minutes to get the supernatant.(Day-1/S1; Day-2/S2; Day-3/S3).Optimization of the three substrates were done with OD600 values in UV spectrometer with three lactic acid bacteria isolated from samples . The Chemical parameters like phosphate in ammonium molybdate method (690nm), Nitrate in Brucine- sulphanilic method(410nm), Total Organic Carbon(TOC) in Walkey Black's method were done in triplicates and the mean was taken as the result. Solid waste S1 was found to be optimum based on OD 600 values and it was chosen for lactic acid production.

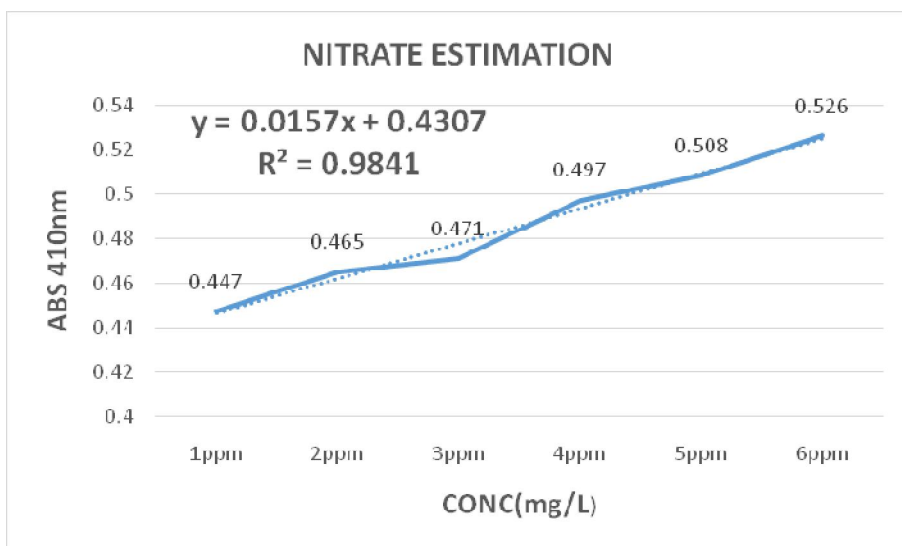
Chemical parameters:



Fig 4. Solid Waste Powder and Acid Hydrolysate



Graph 1. Phosphate Estimation



Graph 2. Nitrate Estimation

Total Organic Carbon(TOC)

Table.4 .TOTALORGANIC CARBON

SAMPLE (triplicate)	TOC%	Std deviation	TRIPLICATE AV
S1	43.8		
S1*	48.7		
S1**	51.6	3.942503	48.03
S2	58.5		
S2*	46.8		
S2**	44.8	7.400225	50.03
S3	40.9		
S3*	37.05		
S3**	44.8	3.875027	40.91



Fig 5. TOC titration endpoint green color

Table.5. Overall results for chemical parameters

SAMPLE	Day1 (S1)	Day2 (S2)	Day3 (S3)
PHOSPHATE(ppm)	7.34	1.054	7.024
NITRATE(mg/l)	229	297	260
TOC(%)	48.03	50.03	40.91

SAMPLE 1 is chosen as the solidwaste optimum substrate based on OD600 values by the LAB strains

The phosphate content in the substrates S1, S2,S3 was found to be 7.34,1.054,7.024 ppm. The nitrate content in the substrates S1, S2,S3 was found to be 229,297,260 mg. The **total organic carbon**TOC content in the substrates S1, S2,S3 was found to be 48.03%, 50.03%, 40.91%.



Fig 6. LA Production in Erlenmeyer's flasks

1.2 Lactic acid Estimation:

Samples were taken from curd, milk, idli batter for this study. **deMan Rogosa Sharpe** MRS agar was prepared according to the standard composition and by serial dilution of the raw samples isolation was done by spread plating in petri dishes. For obtaining pure culture, after incubation for 24-48 hours at 37 degrees, colonies were streak plated in **deMan Rogosa Sharpe** MRS agar two times. Biochemical tests like staining-Gram positive, catalase test-negative, oxidase negative, acid fermentation test –positive, adding 1ml culture to 2ml of 2% ferric chloride solution with yellowish green color identification of iron lactate formation proved the four isolated organisms to be *Lacto bacillus* species.

Inoculum preparation:

Fifty (50) mL of inoculum medium containing nutrient broth 13 g/L, pH 6.5, was transferred to a 250 mL Erlenmeyer flask and was sterilized in an autoclave at 15 lbs/inch² pressure at 121°C for 20 min. After cooling at room temperature, a loopful of freshly grown bacterial culture was aseptically transferred to it. The flask was incubated overnight at 37°C and 150 rpm in a rotary shaking incubator for 24hrs.

Table.6. Optimum parameters of LAB strains:

<u>Growth parameters</u>	<u>Milk</u>	<u>Curd</u>	<u>Idli batter</u>
Initial OD	0.590	0.6	0.666
Initial pH	6.5	6.5	6.5
Optimum temp	37deg	37deg	37deg

Fermentation for lactic acid production:

Lactic acid production was done in airtight conical flasks with 500ml of the broths prepared in duplicates at 37 degrees 150rpm. One (1)mL (2%) of 24 h grown inoculums was transferred to the broth medium with an initial pH 6.5. 1ml of the culture was removed from the tubes and OD 600 values were observed in UV spectro photometer. The optimum substrate was chosen based on OD values as SAMPLE 1 of solid waste collected for lactic acid production. The process was repeated with the three substrates for the three organisms in duplicates .Lactic acid estimation was done at 12hrs,24hrs,48hrs,72 hrs,84hrs,96hrs. By removing 10ml aliquots and centrifuging at 4000rpm 30minutes to remove the cell debris and impurities, the clear supernatant was used for lactic acid estimation in UV spectrometric assay at 390nm.

The growth of *Lactobacillus* genus strainMI23(milk), *Lactobacillus* genus strainCU23(curd), *Lactobacillus* genus strainIB23(idli batter) were found to be approximately 0.6 OD 600 absorbance. That is 0.6×10^8 CFU/ml or 6×10^7 CFU/ml.

UV spectrometric assay at 390nm:

Quantification of lactic acid

Using the spectrophotometric method, LA quantification was performed according to

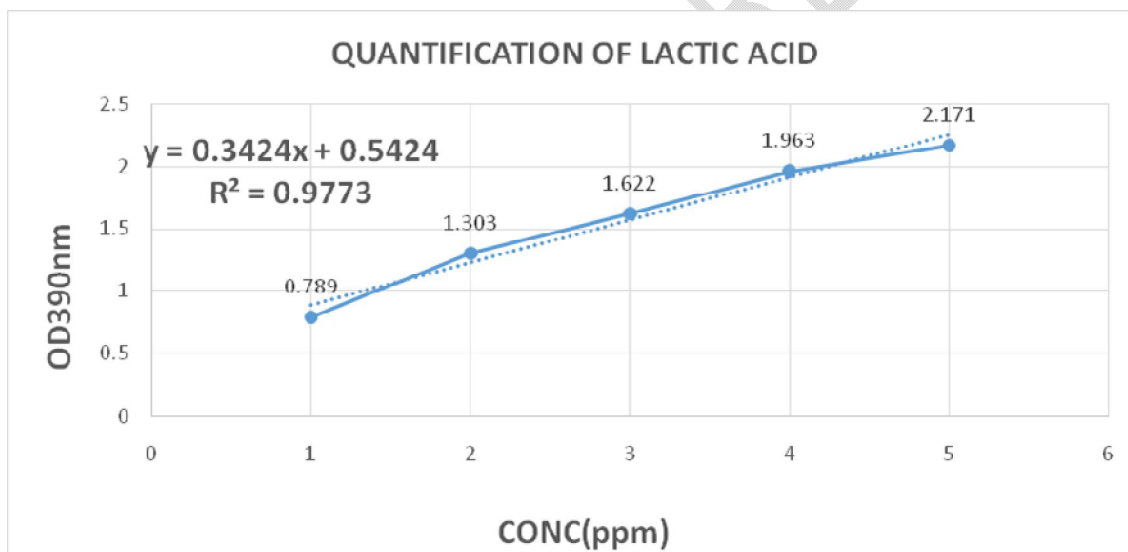
Borshchevskaya et al. [32] Briefly, 1000 µl of the supernatant was added into a 2 ml of 2%FeCl₃ solution (200mg in 100ml distilled water) and mixed it. The Greenish yellow colored product was measured using UV-visible spectrophotometer at OD390 nm within 1-15 min. In here, the reaction of iron (III) chloride with the lactic acid in the aqueous solution results in the formation of iron (III) lactate in yellowish-green color solution.



Lactic acid standard curve was prepared using known concentrations of lactic acid (1ppm, 2ppm, 3ppm, 4ppm, 5ppm) obtained by diluting 1N lactic acid stock solution. A graph was plotted with known concentrations on the Xaxis and absorbance at 390nm on the Y axis. From the standard curve of lactic acid obtained, concentrations of lactic acid in unknown sample were estimated.

Table.7.Standard curve of lactic acid

CONCENTRATION(PPM)	OD390nm
1	0.789
2	1.303
3	1.622
4	1.963
5	2.171



Graph 3.Quantification of Lactic Acid

Table 8 .Lactic acid production in
Solid waste broth

DESCRIPTION	CONC(mg/L)- milk	CONC(mg/L)- curd	CONC(mg/L)- idly batter
12hrs	3232	4896	2017
24hrs	2957	5118	2519
48hrs	3255	4868	2837
72hrs	3024	4771	2650
84hrs	2341	3731	2618
96hrs	1973	3775	1681

Table 9 .Lactic acid production in
Solid waste-tryptic soy(4:1) broth

DESCRIPTION	CONC(mg/L)- milk	CONC(mg/L)- curd	CONC(mg/L)- idly batter
12hrs	1900	2000	1786
24hrs	1748	2618	1960
48hrs	2388	3404	2475
72hrs	4046	4093	2516
84hrs	2104	2352	1830
96hrs	1672	2540	1584

Table10.Lactic acid production in
deMan Rogosa Sharpe MRS broth

DESCRIPTION	CONC(mg/L)- milk	CONC(mg/L)- curd	CONC(mg/L)- idly batter
12hrs	1243	1807	527
24hrs	2754	2808	1234
48hrs	1883	3827	1296
72hrs	2002	4330	2706
84hrs	182	2162	1365
96hrs	89	3071	1310

1.3 Substrate consumption by lactic acid bacteria:

Estimation of glucose in the given solution is done by Anthrone method. Carbohydrates are dehydrated with conc.sulphuric acid to form furfural which condenses to form blue green color complex that can be measured at 620nm in UV spectrometer.

Anthrone reagent-200mg anthrone in 100ml concsulphuric acid.

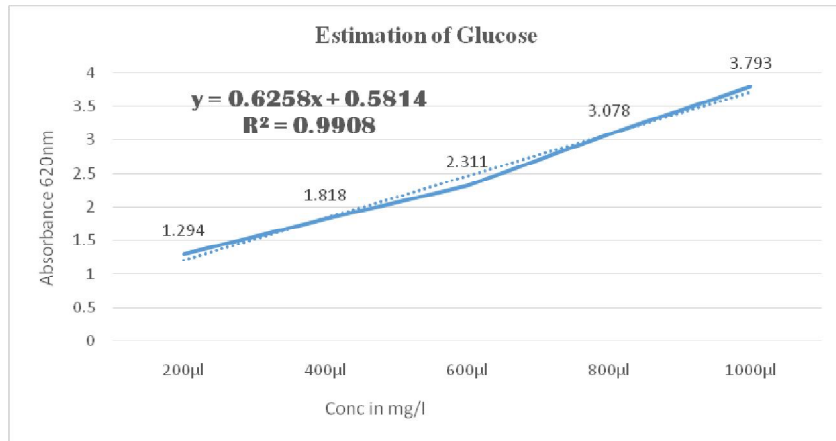
Stock solution-100mg glucose in 100ml distilled water.

0.2ml-1ml of working standard solution of glucose was taken in five test tubes and add water to bring the volume to 1ml in each test tube. Add 4ml anthrone reagent to each test tube and mix the contents well and keep in water bath for 10minutes. Allow the test tubes to cool down and measure the OD620 values. Blank solution was 1ml distilled water with 4ml anthrone reagent

Table 11. Analysis of initial and final levels of glucose(Anthrone method)

standard	abs620nm
200µl(20mg)	1.294
400µl(40mg)	1.818
600µl(60mg)	2.311
800µl(80mg)	3.078
1000µl(100mg)	3.793

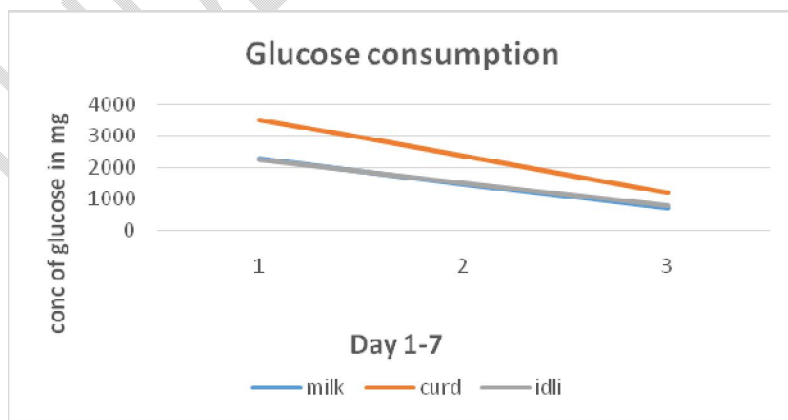
Construct a calibration curve by plotting (20mg-100mg)concentration of glucose on X axis and OD620 values on Y axis. From the graph, concentration of glucose in the unknown sample was estimated and tabulated. Initial values on day 1 and final values of day 6 are estimated in a similar way and the results were tabulated.



Graph 4. Estimation of Glucose

Table 12. Glucose consolidated value

Sample	Initial	Mid value	Final(mg/l)
Milk	2259	1467	676
Curd	3520	2351	1183
Idli	2220	1492	764



Graph 5. Glucose Consumption

Result & Discussion:

All the three strains of *Lactobacillus* were able to utilize the solid waste substrate efficiently. The glucose consumption efficiency of the strains was found to be approximately 65% .

Lactobacillus pentosus B-227 metabolized the most carbohydrate (62%) and produced the highest concentration of lactic acid in AHMSW-Acid hydrolysed municipal solid waste (21.2 mg/mL) containing 41.3 mg/mL carbohydrate[33].

The L-lactic acid yields of 0.18 and 0.19 (g/g biomass) were obtained for corn stover and aspen respectively, while those for seaweeds *Ulva pertusa*, *Laminaria sp.*, and *Gelidium amansii*, were 0.16, 0.17, and 0.17 (g/g biomass)[29].

Lactic acid bacteria were isolated from various sources such as milk, curd, whey, fermented idly dough and pickles. Effect of carbon sources, temperature, pH and inoculum levels on the growth of lactic acid bacteria were investigated. *Lactobacillus delbrueckii* was found to produce 135 gl-1 of lactic acid from 150 gl-1 of glucose followed by *Lactobacillus plantarum* (120 gl-1) and *Lactobacillus casei* (112.5 gl-1). Maximum glucose conversion to lactic acid was observed at process conditions of pH 5.5, temperature 37o C, 10% inoculum level and fermentation period of 72 hours in MRS broth[34]

Lactic acid (LA) fermentation from food waste was investigated by batch fermentation experiments using methanogenic sludge, fresh food waste and anaerobic activated sludge as inocula. *Lactobacillus* was enriched (83.4–98.5%) during the fermentation process, although abundant microbial diversity existed in the initial inocula. The optimal LA concentration (20.7–28.4 g/L) and yield (0.36– 0.46 g/g-TS) were obtained at pH 5 with all three inocula, showing a higher TSS removal rate, substrate degradation rate and microbial enzyme activity[35].

From all these studies we can see that lactic acid production varies depending upon the strain as well as substrate used. **Lactic acid production from solid waste collected is a sustainable approach to convert waste into wealth.** From the data obtained, **lactic acid produced by *Lactobacillus* genus strain MI23(milk), *Lactobacillus* genus**

strainCU23(curd), *Lactobacillus* genus strainIB23(idli batter) in the three substrates in the time interval of 48hrs-72hours were as below:

Lactobacillus genus strainMI23(milk) produced maximum of 4046mg/l in Solid waste: Tryptic Soy (4:1) substrate, 3255mg/l in Solid waste substrate, 2754mg/l in deMan Rogosa Sharpe MRS substrate.

Lactobacillus genus strainCU23(curd) produced maximum of 4093mg/l in Solid waste: Tryptic Soy (4:1) substrate, 4868mg/l in Solid waste substrate, 4330mg/l in deMan Rogosa Sharpe MRS substrate.

Lactobacillus genus strainIB23(idli batter) produced maximum of 2516mg/l in Solid waste: Tryptic Soy (4:1) substrate, 2837mg/l in Solid waste substrate, 2706mg/l in deMan Rogosa Sharpe MRS substrate.

Summary:

-Three isolates were chosen from pure culture isolated in deMan Rogosa Sharpe MRS agar from three samples each one from milk, curd, idli batter.

-Biochemical tests were conducted in triplicates following Bergey's manual of systematic bacteriology. All the three strains were confirmed as *Lactobacillus* genus

***Lactobacillus* genus strainMI23 (milk), *Lactobacillus* genus strainCU23 (curd),**

***Lactobacillus* genus strainIB23 (idli batter).**

Catalase test negative, oxidase test negative, morphology in gram's staining rod shaped bacilli, Gram positive, Acid fermentation test positive with no gas formation were observed for the isolated three strains.

-Solid waste was collected for three consecutive days(approx..5kg) and biodegradable fragment was segregated and dried in hot air oven to remove the moisture content and grinded well to get a fine powder. The chemical parameters like phosphate, nitrate, TOC estimation were done in triplicates.

-Solid waste pretreatment was done with heating in dilute acid and the hydrolysate was obtained after filtration and utilized for lactic acid production.

-Initial and final levels of glucose present in solid waste substrate (S1)were estimated

in Anthrone method to check the consumption ability of the LAB strains.

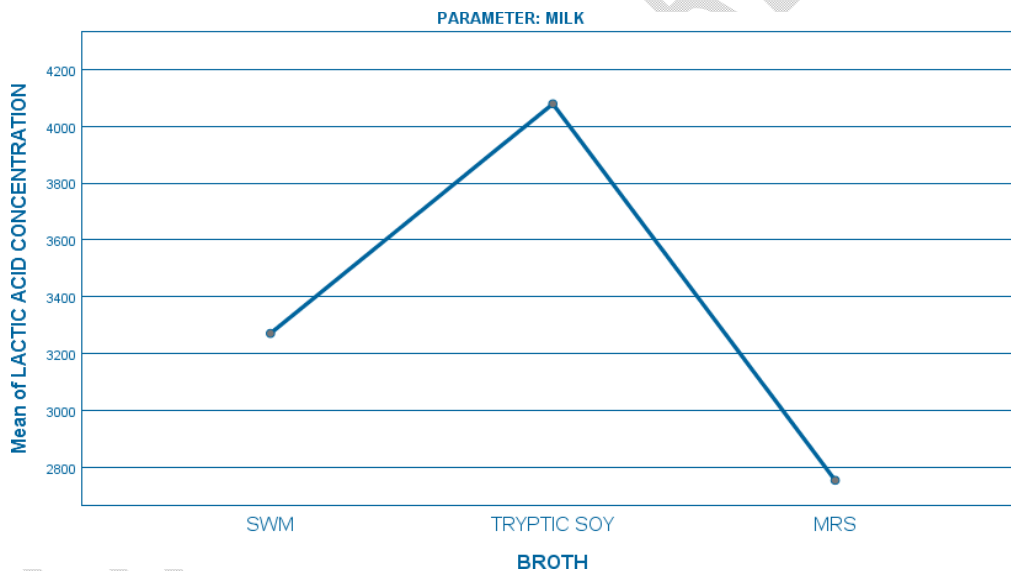
-Lactic acid estimation for time interval 12hrs-96hrs in UV spectrometer OD390nm-
Ironlactate formation was done.

-The results were tabulated and discussion and analysis was done for all the three strains
in all the three substrate combinations chosen.

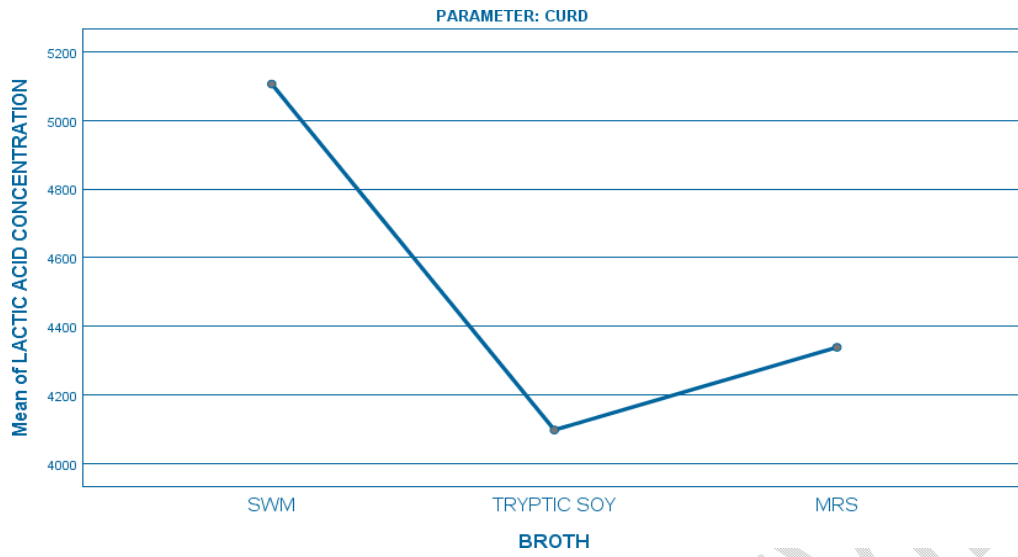
Statistical analysis:

All the experiments were conducted in triplicates and the mean values were taken as
result. The standard deviation values were calculated in Microsoft Excel software. In
SPSS software, data were analyzed in one way Anova with significance ($p < 0.05$).
TukeyHSD was used.

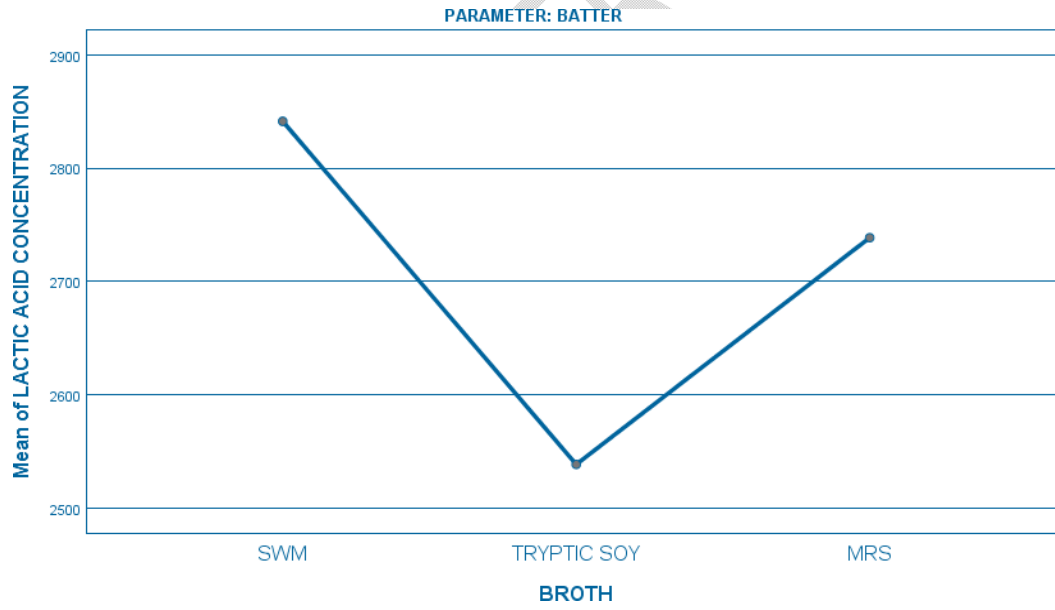
The mean difference is significant at the 0.05 level



Graph 6: Graphical representation of Parameter : Milk



Graph 7: Graphical representation of Parameter : Curd



Graph 8: Graphical representation of Parameter : Idli Batter

2.0 Are *Xanthomonas* sp. cutting edge microbiological tools in minimizing plastic pollution- The Solution:

Out of the domestic solid waste collected 40%-50% is plastic waste. Plenty of plastic remains enter into our landfills after major fraction is recycled. The bacterial strain isolated from the intestine of a Japanese carpenter bee (*Xylocopa appendiculata*) was analyzed by giving polyurethane as sole carbon source. The bioconversion and degradation abilities of the strain to degrade polyacrylic-, polyester-, and polyether-based PU were characterized by weight loss measurement, SEM, FTIR, chemical composition analysis. The strain was identified as *Xanthomonas* sp. HY-71 and the exopolysaccharide yields with acryl PU-Siegel and PS-PU foam as nutritional source were found to be 24.6 g/L and 22.6 g/L [36].

Xanthan gum is a water-soluble exo-polysaccharide that has wider industrial applications in food, agriculture, oil, paint and cosmetics. It is produced industrially from carbon sources by fermentation using the gram-negative bacterium *Xanthomonas campestris*. Various cheap raw materials are used as substrate to produce xanthan gum by fermentation method using bacteria and yeast. There are plenty of literature suggesting production of xanthan gum from potato crop residue, cassava bagasse, tapioca pulp, spent coffee biomass and agro industrial waste [37]. Xanthan is the most commercially produced industrial gum, obtained by fermentation of glucose by *Xanthomonas campestris*, with an annual worldwide production of 30,000 tons, which corresponds to a market of \$408 million [38].

Conclusion:

Disposal of municipal solid waste is a huge and mammoth task to be handled in cities like Coimbatore. Biodegradable fragment is nearly 50% of the waste generated. Biomanure production from solid waste is the traditional practice in our municipalities. Production of more valuable product like lactic acid from solid waste is a sustainable and greener way of disposal of waste. The primary use of lactic acid is in food industry as a preservative and flavoring agent. Polymerization of lactic acid gives polylactic acid that is treated as biopolyester having applications as a packaging material. Ethyl lactate an ester of lactic acid is a green solvent for wide range of chemicals. In domestic solid waste, approximately 40% is plastic waste out of which half is recycled and balance remains as mountains in our dumpsites and landfills. This is due to the usage of plastic containers in the form of pet bottles, containers, disposable covers and everything we are packing in the form of nondegradable plastic. Plastic pollution has become a major threat to marine living organisms and terrestrial organisms like cattle, birds due to ingestion of microplastics leading to their death. Plastic has become an inevitable partner in our modern life style thus accumulating mountains of plastic waste. There is no doubt in the fact that usage of plastic in daily life is very convenient and economical due to its low cost. But it is hazardous to our land and water resources. Hence switching to bioplastics that are easily degradable is the only way to save our environment. We have to bring down the cost of bioplastics(PLA) to the cost comparable to traditional plastic. Production of lactic acid, precursor of poly lactic acid PLA from solid waste is a

sustainable & greener method to dispose waste. Bioconversion of plastic into pseudo plastic-xanthan gum is a promising tool to minimize plastic pollution.

References

1. Reuben, R. C., et al. "Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties." *Journal of dairy science* 103.2 (2020): 1223-1237.
2. Spano, Giuseppe, and Salvatore Massa. "Environmental stress response in wine lactic acid bacteria: beyond *Bacillus subtilis*." *Critical reviews in microbiology* 32.2 (2006): 77- 86.
3. Ahmad, Ashfaq, et al. "An overview of biodegradable poly (lactic acid) production from fermentative lactic acid for biomedical and bioplastic applications." *Biomass Conversion and Biorefinery* (2022): 1-20.
4. Jiménez, L., et al. "Polylactic acid (PLA) as a bioplastic and its possible applications in the food industry." *J Food SciNutr* 5.2 (2019): 2-6
5. Bátori V et al (2018) Anaerobic degradation of bioplastics: A review. *Waste Management* 80:406–413.
6. Tullo ,Alex. 2000. "Plastic found at the end of the maize". *Chem Engi News* **78**(3): 13.
7. Groot W, Krieken JV, Sliekersl O, Vos DS. 2011. Production and purification of lactic acid and lactide. In: RA Auras, L-T Lim, SEM Selke, H Tsuji, editors. *Poly(lactic acid): synthesis, structures, properties, processing, and applications*. Chichester: Wiley. p. 43– 55.
8. Jessen B. 2007. Sustainability and emerging topics in food research and education. *Danish Dairy Food Ind* **17**: 22– 3.
9. Mutsuga M, Kawamura Y, Tanamoto K. 2008. Migration of lactic acid, lactide and oligomers from polylactide food-contact materials. *Food Add Contam* **25**(10): 1283– 90.
10. Ahmed J, Varshney SK, Zhang JX, Ramaswamy HS. 2009. Effect of high pressure treatment on thermal properties of polylactides. *J Food Eng* **93**(3): 308– 12.
11. Raghavan D, Emekalam A. 2001. Characterization of starch/polyethylene and starch/polyethylene/poly(lactic acid) composites. *Polym Degrad Stab* **72**(3): 509– 17.

12. Ke T, Sun X. 2003. Melting behavior and crystallization kinetics of starch and poly(lactic acid) composites. *J Appl Polym Sci* **89**(5): 1203– 10.
13. Yew GH, Mohd Yusof AM, Mohd Ishak ZA, Ishiaku US. 2005. Water absorption and enzymatic degradation of poly(lactic acid)/rice starch composites. *Polym Degrad Stab* **90**(3): 488– 500.
14. Auras R, Singh SP, Singh JJ. 2005. Evaluation of oriented poly(lactide) polymers with existing PET and oriented PS for fresh food service containers. *Packag TechnolSci* **18**(4): 207– 16.
15. Conn RE, Kolstad JJ, Borzelleca JF, Dixler DS, Filer LJ, Ladu BN, Pariza MW. 1995. Safety assessment of polylactide (PLA) for use as a food-contact polymer. *Food Chem Toxicol* **33**(4): 273– 83.
16. Tawakkal, Intan SMA, et al. "A review of poly (lactic acid) based materials for antimicrobial packaging." *Journal of food science* 79.8 (2014): R1477-R1490.
17. Szafranska W. 2012. Natural controlled-releasing antifungal film ANTIPACKTMAF. <http://www.handary.com/news/show/?id=10001>. Accessed: 2013 July 3
18. Guarda, Abel, et al. "The antimicrobial activity of microencapsulated thymol and carvacrol." *International journal of food microbiology* 146.2 (2011): 144-150.
19. Jin T, Niemira BA. 2011. Application of polylactic acid coating with antimicrobials in reduction of *Escherichia coli* O157:H7 and *Salmonella stanley* on apples. *J Food Sci* **76**(3): 184– 8.
20. Bhatia A, Gupta RK, Bhattacharya SN, Choi HJ. 2007. Compatibility of biodegradable poly(lactic acid) (PLA) and poly(butylene succinate) (PBS) blends for packaging application. *Korea-Aust Rheol J* **19**(3): 125– 31.
21. Suyatma NE. 2004. Mechanical and barrier properties of biodegradable films made from chitosan and poly(lactic acid) blends. *J Polym Environ* **12**(1): 1– 6.
22. Djukić-Vuković, Aleksandra, et al. "Towards sustainability of lactic acid and polylactic acid polymers production." *Renewable and Sustainable Energy Reviews* 108 (2019): 238- 252.

23. Zapaśnik, Agnieszka, Barbara Sokołowska, and Marcin Bryła. "Role of lactic acid bacteria in food preservation and safety." *Foods* 11.9 (2022): 1283.
24. Bernard, D., N. Jeyagowri, and T. Madhujith. "Characterization of lactic acid bacteria isolated from idli batter and their susceptibility to antibiotics." *Tropical Agricultural Research* 32.4 (2021): 453-461.
25. Coelho, Márcia C., Francisco Xavier Malcata, and Célia CG Silva. "Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions." *Foods* 11.15 (2022): 2276.
26. Yilmaz, Birsen, et al. "Recent developments in dairy kefir-derived lactic acid bacteria and their health benefits." *Food Bioscience* 46 (2022): 101592.
27. Hossain, Tanim Jabid, et al. "Antipathogenic action and antibiotic sensitivity pattern of the borhani-associated lactic acid bacterium *Weissella confusa* LAB-11." *Antipathogenic Action and Antibiotic Sensitivity Pattern of the Borhani-Associated Lactic Acid Bacterium Weissella confusa LAB-11 (July 13, 2022)* (2022).
28. Ghosh, Tamoghna, et al. "Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products." *Frontiers in microbiology* 10 (2019): 502.
29. Al-Hindi, Rashad R., and Salem Abd El Ghani. "Production of functional fermented milk beverages supplemented with pomegranate peel extract and probiotic lactic acid bacteria." *Journal of Food Quality* 2020 (2020).
30. Hwang, H.J., Lee, S.Y., Kim, S.M. et al. Fermentation of seaweed sugars by *Lactobacillus* species and the potential of seaweed as a biomass feedstock. *Biotechnol Bioproc E* (2011) 16: 1231. <https://doi.org/10.1007/s12257-011-0278-1>.
31. Costa, Stefania, et al. "Fermentation as a strategy for bio-transforming waste into resources: lactic acid production from agri-food residues." *Fermentation* 7.1 (2020): 3.
32. Borshchevskaya, L. N., Gordeeva, T. L., Kalinina, A. N., & Sineokii, S. P. (2016) Spectrophotometric determination of lactic acid. *Journal of analytical chemistry*, 71(8), 755-758.
33. McCaskey, Thomas A., et al. "Bioconversion of municipal solid waste to lactic acid by *Lactobacillus* species." *Applied biochemistry and biotechnology* 45 (1994):

555-568.

34. A.Sheeladevi, and N. Ramanathan. "Lactic acid production using lactic acid bacteria under optimized conditions." *Int. J. Pharm. Biol. Arch* 2.6 (2011).
35. Tang, Jialing, et al. "Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula." *Bioresource technology* 224 (2017): 544- 552.
36. Kim, Jong-Hoon, et al. "Biodegradation of polyurethane by Japanese carpenter bee gut associated symbionts *Xanthomonas* sp. HY-71, and its potential application on bioconversion." *Environmental Technology & Innovation* 28 (2022): 102822.
37. Aarthy Palaniraj, and Vijayakumar Jayaraman. "Production, recovery and applications of xanthan gum by *Xanthomonas campestris*." *Journal of food engineering* 106.1 (2011): 1-12.
38. Kalogiannis, S., Gesthimani, I., Maria, L.K., Dimitrios, A.K., George, N.S., 2003. Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. *Process Biochemistry* 9, 249–256