Pivotal role of microbes in solid waste management

Abstract

Lactic acid bacteria 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. In this study, lactic acid bacteria were isolated from samples of milk, curd, idly batter and screened for lactic acid production. Three isolates were chosen for the study each one from milk, curd, idly 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 combinations of substrate were chosen for production and the results were compared. Disposal of solid waste in economical and greener way is a smart method of saving environment. Bioconversion of plastic into pseudoplastic by *Xanthomonas sp.(*through exopolysaccharide pathway) are greener techniques to crush down the accumulated plastic mountains to ground level.

Comment [MM1]: Three different substrate

Comment [MM2]: Authors should mention the basic methodology used and present the results obtained from studies

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. **Reuben et al., (2020)** 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.*Lactobacilluscasei*, *Lactobacillusplantarum* (which produced plantaricin EF), *Lactobacillus fermentum*, and *Lactobacillus paracasei* were identified as the four most promising LAB strains with probiotic potential.**Spano et al.,(2006)** 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, Tetragenococcus, 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.

Poly(lactic 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. **(Ahmad etal.,2022)** 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. **(Jimenez et al.,2019)**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**. (Batori et al.,2018)**The 3 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.

Table 1 Use of PLA as a Packaging material

PLA as a Packaging material	**Literature**
Food and beverage containers, cups, overwrap, blister packages, coated paper and board	Tullo 2000; Groot 2011
Yoghurt cups	Jessen2007
Production of lunch boxes and fresh food packaging	Mutsuga2008
Containers for packaging of bottled water, bottled juices and yogurts	Ahmed2009
Fully renewable and degradable packaging materials	Raghavan and Emekalam2001
Fully renewable and degradable packaging materials	Ke and Sun 2003; Suyatma 2004;
Fully renewable and degradable packaging materials	Yew and others 2005;Bhatia 2007
Commercial thermoplastics such as PET	Auras2005
Food packaging including direct contact applications	Conn1995
Antimicrobial PLA releasing chitosan containing natamycin onto the surface of solid foods such as cheese, fruits, vegetables, meat, and poultry	Szafranska2012
PLA based antimicrobial bioplastics	Guarda 2011; Jin and Niemira 2011
Compost bags, disposable bags	Bhatia et al.,2007
Biodegradable film blends of chitosan with poly(lactic acid) (PLA)	Suyatma et al.,2004
LA biopolymers as biomedicine stunts	Djukie-vukovie et al.,2019

Comment [MM3]: Not in reference section

History of fermented food products:

(Zapaśnik et al.,2022) 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. **(Bernard et al.,2021)**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 9 the fermentation period of 0 to 32 hours, the pH value steadily decreased from 6.28 to 3.72, while titratable acidity increased from 0.24 to 0.92%. All of the isolates were Gram-positive, nonmotile, spore-free, and catalase negative. The biochemical and sensory properties of idlibatter were altered by fermentation. The flavor, texture, and nutritional value of fermented foods are increased. **(Coelho et al.,2022**)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. **(Yilmaz et al.,2022**)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. **(Hossain et al.,2022**)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 confusestrain LAB-11can 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.**(Ghosh et al.,2019)**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). **(Al-Hindi et al.,2020**)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.

(**Hwang et al.,2011**)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. (**Costa et al.,2020**) 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

Comment [MM4]: Authors should reconsider this statement

Comment [MM5]:

Comment [MM6]: Consider spelling

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 ± 0.3% to 46.0 ± 0.7% have been obtained.

1.0 Lacto bacillus sp. as solid waste degraders

Methodology involves Isolation and screening for lactic acid bacteria from milk, curd, idly batter samples. Serial dilution, spread plating, streak plating in MRS agar ~~are~~weredone to obtain pure culture of lactic acid bacteria. Biochemical tests like gram staining, catalase, oxidase, acid fermentation ~~were~~wasdone.

Comment [MM7]: Reconcile spelling idli or idly

Comment [MM8]: Full meaning before acronym

Fig1.Acid Fermentation Yellow Color by LAB

Table.2 MorphologicalIdentification oftheColonies:

Description	milk(m)	curd-c	Idlybatter(I)
Sizeofthecolony(mm)	0.5-1mm	0.5-1mm	0.5-1mm
Size appearance	medium	Large	small
Formofthecolony	circular	circular	circular
Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Color	White	White	White

Comment [MM9]:

Gramstainingcolor	**Purple**	**Purple**	**Purple**
Gramstaining	**positive**	**positive**	**Positive**

AccordingtoBergey'smanualofsystematicbacteriology,fromtheresultsobtained,isolates from milk(m), curd©, idly batter(I) were confirmed as ***Lactobacillus sp***.

Comment [MM10]:

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

Description	**Milk(m)**	**Curd-c**	**IdlyBatter(I)**
Shape	rodshaped	rodshaped	rodshaped
Gramstaining	positive	positive	positive
Catalase	negative	negative	negative
Oxidasetest	negative	negative	negative
Acidfermentation	positive	positive	positive
Gasbubbleformation	negative	negative	negative

Fig2.LacticAcid Bacteria fromMilk, Curd,Idly Batterin MRSAgar

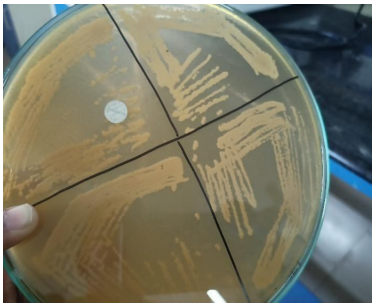


Fig-3 Streak plating of lactobacillus sp.

1.1 Substratepretreatment:

SolidwastewascollectedforthreedaysinResidentialareaofRathinapuri,Coimbatore weighing approximately 5kg/day. The solid waste collected was segregated andbiodegradable fragment was processed in hot air oven at 80degrees till the moisture wasevaporated. Then the dried matter wasgrinded well to obtainfine particle. Solid wastehydrolysate was prepared by treating 5gm accurately weighed solid waste powder(optimumsolid waste dosage) with 100ml of 0.1N sulphuric acid**(5%).** The mixture was heated at 70degrees for 20minutes in hot plate in open beaker. The mixture was allowed to cool to roomtemperatureandcentrifugedat5000rpm-15minutestogetthesupernatant.(Day-1/S1;Day-2/S2; Day-3/S3).Optimization of the three substrates were done with OD600 values in UVspectrometer with three lactic acid bacteria isolated from samples . The Chemical parameterslikephosphateinammoniummolybdatemethod(690nm),NitrateinBrucine-sulphanilicmethod(410nm), Total Organic Carbon(TOC) in Walkey Black's method weredone in triplicates and the mean was taken as the result. Solid waste S1 was found to beoptimumbased on OD600 valuesand it was chosen forlacticacid production.

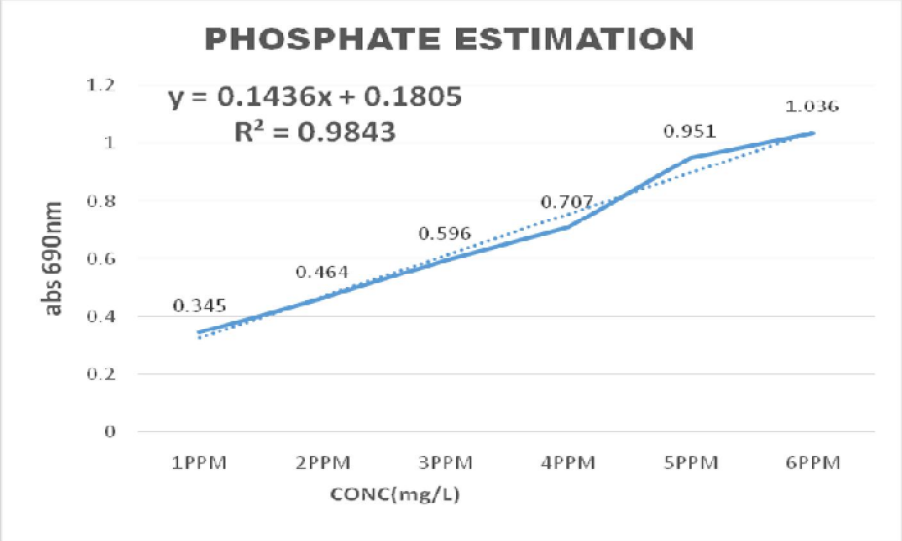
Comment [MM11]: Indicate numbers of samples collected

Comment [MM12]: 80℃or °

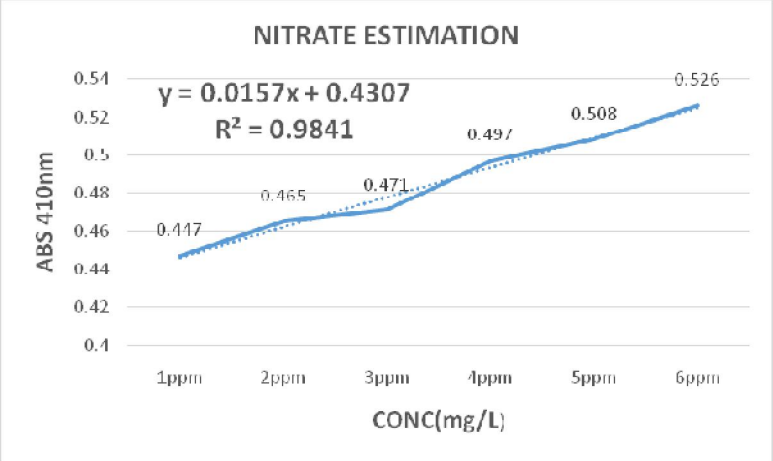
Comment [MM13]: Celsius or Fahrenheit

Chemicalparameters:

Fig4.SolidWastePowderand AcidHydrolysate



Graph1. PhosphateEstimation



Graph2.NitrateEstimation

Total Organic Carbon(TOC)

Table.4 .TOTALORGANICCARBON

SAMPLE	TOC%	Std deviation	TRIPLICATEAV
S1	43.8		
S2	48.7		
S3	51.6	3.942503	48.03
S4	58.5		
S5	46.8		
S6	44.8	7.400225	50.03
S7	40.9		
S8	37.05		
S9	44.8	3.875027	40.91

Comment [MM14]: Why 8 samples here

Fig5. TOC titration endpoint green color

Table.5. Overall results forchemical parameters

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

Comment [MM15]: Why 3 sample here?

SAMPLE1ISCHOSENASTHESWOPTIMUMSUBSTRATEbasedonOD600 valuesbytheLABstrains

Comment [MM16]: Define before acronym

The phosphate content in the substrates S1, S2,S3 was found to be 7.34,1.054,7.024ppm. The nitrate content in the substrates S1, S2,S3 was found to be 229,297,260 mg. TheTOCcontent in thesubstratesS1, S2,S3 was foundto be48.03%,

50.03%,40.91%.

Fig6. LA Production in Erlenmeyer's flasks

1.2 Lactic acid Estimation:

~~Three~~Sampleswereta ken~~forstudy like~~fromcurd,milk,idly batterfor this study.MRSagarwasprepared according to the standard composition and by serial dilution of the raw samples, isolationwasdonebyspreadplatinginpetridishes.Forobtainingpureculture,afterincubation for 24-48 hrs at 37 degrees, colonies were streak plated in MRS agar two times.Biochemical tests like staining-Gram positive, catalase test-negative, oxidase negative, acidfermentation test – positive, adding 1ml culture to 2ml of 2%ferric chloride solution withyellowishgreencoloridentificationofironlactateformationprovedthefourisolatedorganismst o be***Lacto bacillus*species.**

Inoculumpreparation:

Fifty (50) mL of inoculum medium containing nutrient broth 13 g/L, pH 6.5, was transferredto a 250 mL Erlenmeyer flask and was sterilized in an autoclave at 15 lbs/inch^2 pressure at121°C for 20 min. After cooling at room temperature, a loopful of freshly grown bacterialculture was aseptically transferred to it. The flask was incubated overnight at 37°C and150 rpm in arotaryshakingincubator for24hrs.

Table.6.Optimumparameters ofLABstrains:

Growth parameters	Milk	CURD	Idlybatter
InitialOD	0.590	0.6	0.666
InitialpH	6.5	6.5	6.5
Optimumtemp	37deg	37deg	37deg

Fermentation forlacticacid production:

Lactic acid production was done in airtight conical flasks with 500ml of the broths preparedinduplicatesat37degrees150rpm.One(1) mL**(2%)**of24 hgrowninoculumswastransferred to the broth medium with an initial pH 6.5. 1ml of the culture was removed fromthetubesandOD600valueswereobservedinUVspectrophotometer.Theoptimumsubstrate was chosen based on OD values as SAMPLE 1 of solid waste collected forlacticacidproduction.Theprocesswasrepeatedwiththethreesubstratesfor thethreeorganismsinduplicates.Lacticacidestimationwasdoneat12hrs,24hrs,48hrs,72 hrs,84hrs,96hrs.Byremoving10mlaliquotsandcentrifugingat4000rpm30minutestoremovethecell debrisandimpurities,theclearsupernatantwasusedforlacticacidestimation in UVspectrometricassayat 390nm.

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

UVspectrometricassayat390nm:

Quantificationoflacticacid

Usingthespectrophotometricmethod, LAquantificationwasperformedaccordingto

Borshchevskaya et al. (2016). Briefly, 1000 µl of the supernatant was added into a 2 ml of2%FeCl3 solution (200mg in 100ml distilled water)and mixed it. The Greenishyellowcolored product was measured using UV-visiblespectrophotometer at OD390 nm within 1-15min. In here, the reaction of iron (III) chloride withthe lactic acid in the

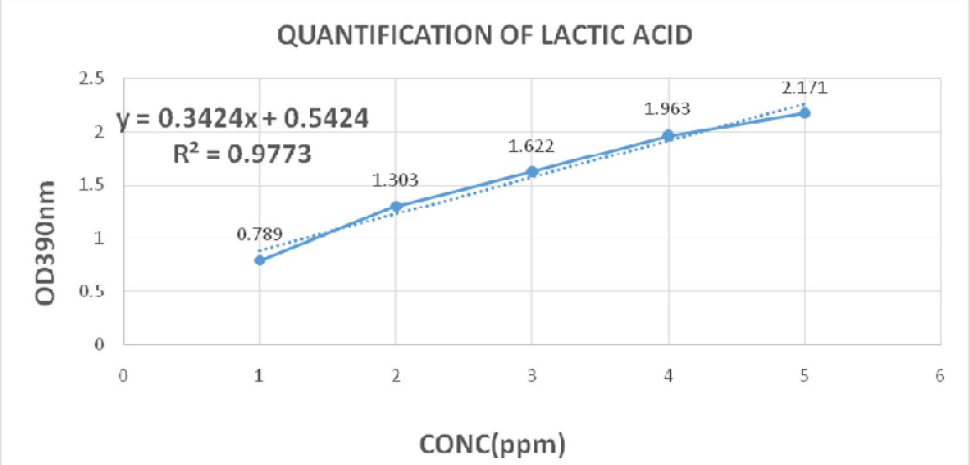
aqueous solutionresultsin theformation of iron(III)lactate inyellowish-greencolor solution.

FeCl3+LacticAcid ⟶ Fe(III)lactate

Lacticacidstandardcurvewaspreparedusingknownconcentrationsoflacticacid(1ppm,2ppm,3ppm,4ppm,5ppm)obtainedbydiluting1Nlacticacidstocksolution.Agraphwas plottedwithknownconcentrationsontheXaxisandabsorbanceat390nmontheYaxis.From the standard curve of lactic acid obtained, concentrations of lactic acid in unknown samplewereestimated.

Table.7.Standardcurveoflacticacid

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



Graph3.Quantificationof LacticAcid

Table 8 .Lactic acid production in SWM broth

Comment [MM17]: Write full meaning

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 SWM-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 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**

Comment [MM18]: Full meaning

1.3 Substrateconsumptionbylacticacidbacteria:

Estimation of glucose in the given solution is done by Anthrone method. Carbohydrates aredehydrated with conc.sulphuric acid to form furfural which condenses to form blue greencolorcomplexthat can bemeasured at620nm in UV spectrometer.

Anthrone reagent-200mg anthrone in 100ml concsulphuric

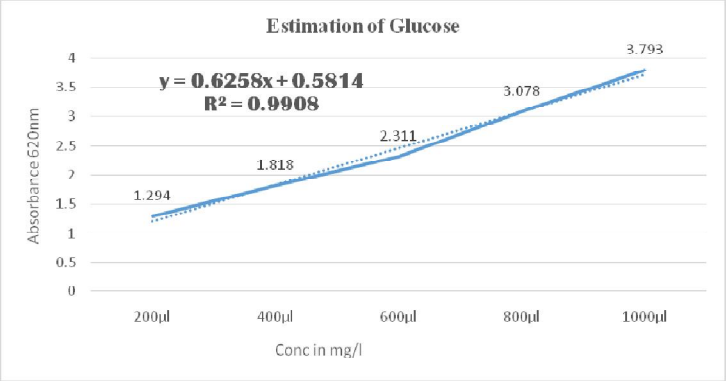
acid.Stocksolution-100mgglucosein 100ml distilledwater.

0.2ml-1ml of working standard solution of glucose was taken in five test tubes and add waterto bring the volume to 1ml in each test tube. Add 4ml anthrone reagent to each test tube andmixthe contentswelland keep inwater bathfor 10minutes. Allow thetesttubes tocooldown and measure the OD620 values. Blank solution was 1ml distilled water with 4mlanthronereagent

Table11.Analysisof initialandfinallevelsof 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**

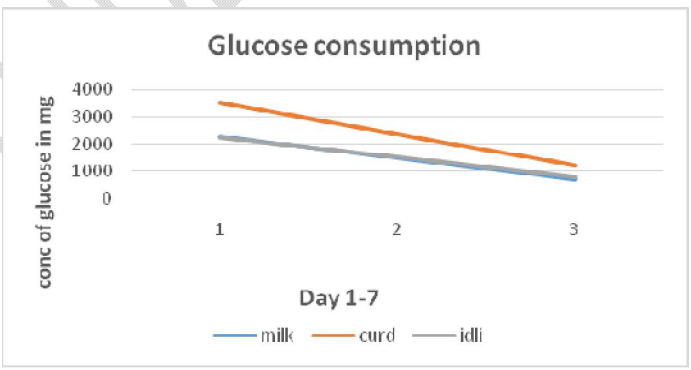
Construct a calibration curve by plotting (20mg-100mg)concentration of glucose on X axisand OD620 values on Y axis. From the graph, concentration of glucose in the unknownsample was estimated and tabulated. Initial values on day 1 and final values of day 6 areestimatedin a similar wayand theresults weretabulated.



Graph4.Estimationof Glucose

Table 12.Glucoseconsolidatedvalue

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



Graph5.GlucoseConsumption

Result&Discussion:

All the three strains of *Lactobacillus* were able to utilize the solid waste substrateefficiently. The glucose consumption efficiency of the strains was found to be approximately65%.

(**McCaskey,etal.,1994**)*Lactobacilluspentosus*B-227metabolizedthemostcarbohydrate(62%) and produced the highest concentration of lactic acid in AHMSW-Acid hydrolysedmunicipalsolid waste (21.2mg/mL) containing41.3mg/mLcarbohydrate.

(**Hwanget al.,2011**)The L-lactic acid yields of 0.18 and 0.19 (g/g biomass) were obtained forcorn stover and aspen respectively, while those for seaweeds*Ulva pertusa*, *Laminaria sp.*,and*Gelidiumamansii*,were0.16, 0.17,and 0.17 (g/gbiomass).

(**Sheeladevi et al.,2011**)Lactic acid bacteria were isolated from various sources such as milk,curd, whey, fermented idly dough and pickles. Effect of carbon sources, temperature, pH andinoculumlevelsonthegrowthoflacticacidbacteriawereinvestigated.*Lactobacillusdelbrueckii*wasfoundtoproduce135gl-1oflacticacidfrom150gl-1ofglucosefollowedby *Lactobacillus plantarum* (120 gl-1) and *Lactobacillus casei*(112.5 gl-1). Maximumglucose conversion to lactic acid was observed at process conditions of pH 5.5, temperature37oC, 10%inoculum level and fermentationperiod of72 hours in MRSbroth

Comment [MM19]: Check reference section and reconcile

(Tang et al.,2017)Lactic acid (LA) fermentation from food waste was investigated by batchfermentationexperimentsusingmethanogenicsludge,freshfoodwasteandanaerobicactivatedsludgeasinocula.*Lactobacillus*wasenriched(83.4–98.5%)duringthefermentation process, although abundant microbial diversity existed in the initial inocula. Theoptimal LA concentration (20.7–28.4 g/L) and yield (0.36– 0.46 g/g-TS) were obtained at pH5 with all three inocula, showing a higher TSS removal rate, substrate degradation rate andmicrobialenzymeactivity.

From all these studies we can see that lactic acid production varies depending upon the strainaswellassubstrateused.**Lacticacidproductionfromsolidwastecollectedisasustainable approach to convert waste into wealth.** From the data obtained**, lactic**

acidproducedby*Lactobacillus*genusstrainMI23(milk),*Lactobacillus*genusstrainCU23 (curd), *Lactobacillus* genus strainIB23(idly batter) in the three substrates inthetimeinterval of48hrs-72hrs wereas below:

Lactobacillus genus strainMI23(milk) produced maximum of 4046mg/l in SWM: Tryptic Soy(4:1)substrate, 3255mg/l in SWMsubstrate, 2754mg/l in MRSsubstrate.

Lactobacillus genus strainCU23(curd) produced maximum of 4093mg/l inSWM: TrypticSoy(4:1) substrate , 4868mg/linSWM substrate, 4330mg/l inMRS substrate.

*Lactobacillus*genusstrainIB23(idlybatter)producedmaximumof2516mg/linSWM:Tryptic Soy(4:1) substrate,2837mg/l inSWM substrate,2706mg/l in MRS substrate.

Summary:

- ThreeisolateswerechosenfrompurecultureisolatedinMRSagarfromthreesampleseachonefrom milk, curd, idlybatter.

- BiochemicaltestswereconductedintriplicatesfollowingBergey'smanualofsystematicbacteriol ogy.All thethreestrainswereconfirmedas**Lactobacillus genus**

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

***Lactobacillus*genusstrainIB23 (idlybatter).**

Catalase test negative, oxidase test negative, morphology in gram's staining rod shapedbacilli, Gram positive, Acid fermentation test positive with no gas formationwereobservedfortheisolated threestrains.

- Solidwastewascollectedforthreeconsecutivedays(approx..5kg)andbiodegradablefragment wassegregatedanddriedinhotairoventoremovethemoisturecontentandgrinded well to get a fine powder. The chemical parameters like phosphate, nitrate, TOCestimationweredonein triplicates.

- Solidwastepretreatmentwasdonewithheatingindiluteacidandthehydrolysatewasobtainedafter filtration and utilized forlacticacidproduction.

-

Initialandfinallevelsofglucosepresentinsolidwastesubstrate(S1)wereestimatedinAnthroneme thod to check the consumption abilityof the LABstrains.

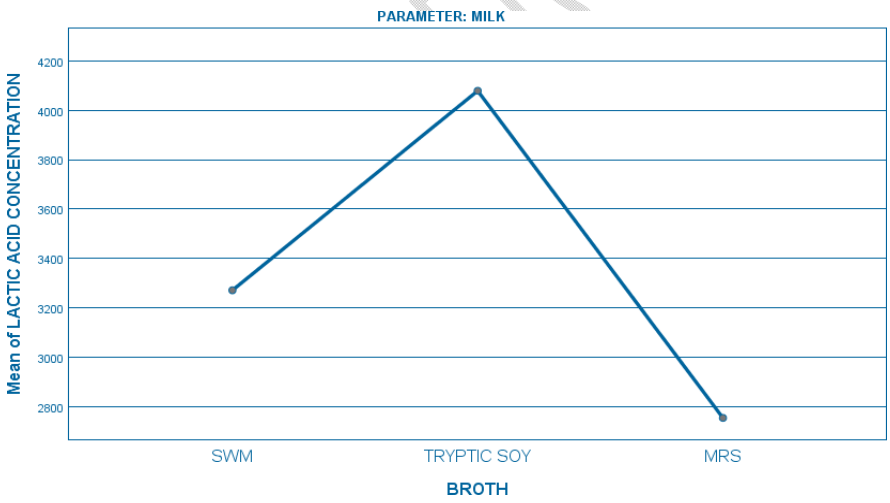
-Lacticacidestimationfortimeinterval12hrs-96hrsinUVspectrometerOD390nm-Ironlactateformation was done.

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

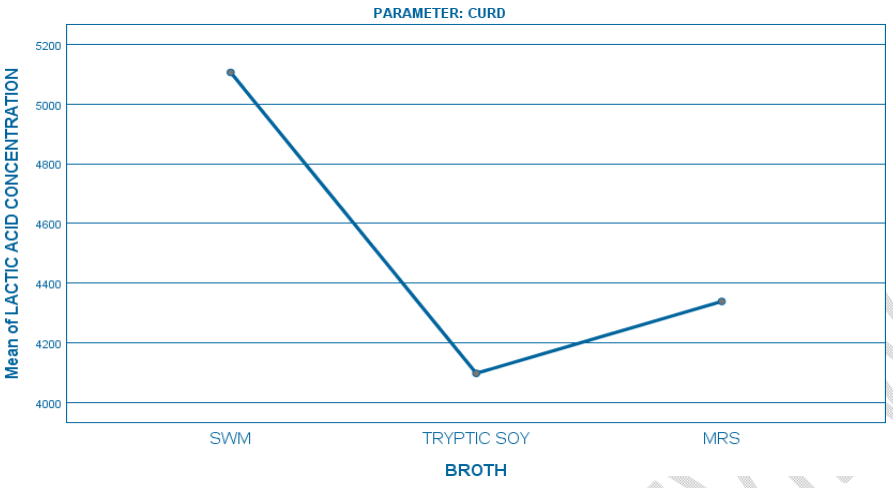
Statisticalanalysis:

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,datawereanalyzed in onewayAnovawith significance($p<0.05$).TukeyHSD was used.

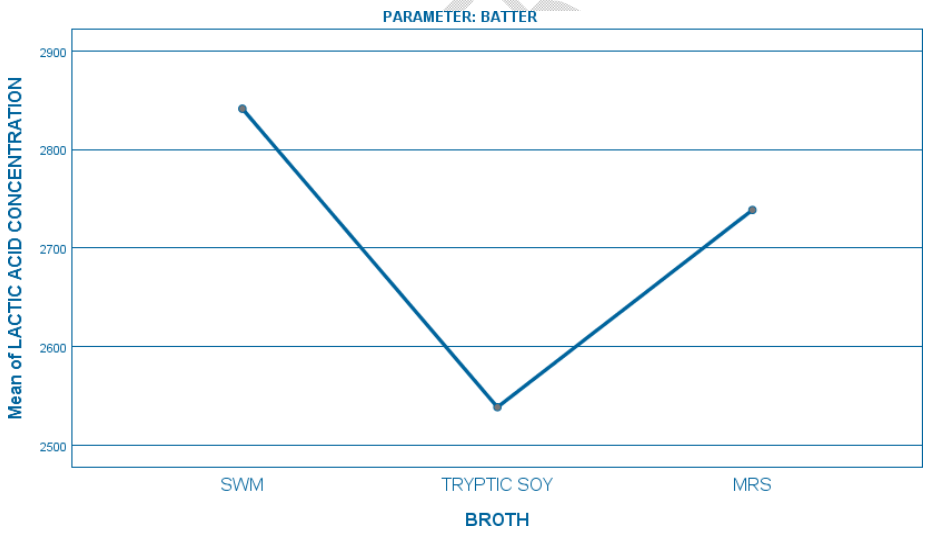
Themeandifferenceissignificantatthe0.05level



6 Graphicalrepresentationof Parameter:Milk



7 Graphical representationofParameter:Curd



8 Graphical representation ofParameter: IdlyBatter

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

Out of the domestic solid waste collected 50% is plastic waste. Plenty of plastic remains enter ~~in to~~intoour landfills after major fraction is recycled. **(Kim, Jong-Hoon, et**

al.,2022) 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 .

(Palaniraj et al.,2011)

Comment [MM20]: Not in reference section. check and reconcile

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 .

(Kalogiannis et al., 2003)

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.

Conclusion:

Disposal of municipal solid waste is a huge and mammoth task to be handled in cities like Coimbatore.Biodegradablefragmentisnearly50%ofthewastegenerated.Biomanure production from solid waste is the traditional practice in our municipalities. Production ofmore valuable product like lactic acid from solid waste is a sustainable and greener way ofdisposalof waste. We can utilize solid waste as raw material for enzymes production like laccase,cutinase, phenol oxidase to degrade plastic. Solid waste can be used as breeding ground for wax worms to degrade plastic in a contained environment like silkworm breeding.

Plasticpollutionhasbecomeamajorthreattomarinelivingorganismsandterrestrialorganisms like cattle, birds due to ingestion of microplastics leading to their death. Most ofthe ($3/4^{th}$)of the plastic waste is recycled. But the remaining ($1/4^{th}$)plastic waste itself remainsas mountains in our dumpsites and landfills. This is due to the usage of plastic containers inthe form of pet bottles, containers, disposable covers and everything we are packing in theform of nondegradable plastic. Plastic has become an inevitable partner in our modern lifestyle thus accumulating mountains of plastic waste. There is no doubt in the fact that usage ofplastic in daily life is very convenient and economical due to its low cost. But it is hazardousto our land and water resources. Hence switching to bioplastics that are easily degradable isthe only way to save our environment. We have to bring down the cost of bioplastics(PLA) tothe cost comparable to traditional plastic. Production of lactic acid, precursor of PLA fromsolidwaste is a sustainable &greenermethod to dispose waste.

Comment [MM21]: Conclusion should be in accordance to the objectives of your research findings

Bioconversion of plastic into pseudo plastic-xanthan gum is a promising tool to minimize plastic pollution.

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Comment [MM22]: Not in text

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Comment [MM26]: Not in text

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