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Application of Solid State Fermentation Technology in Environmental Cleanup and Lactic Acid Production

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Abstract

Solid state fermentation technology utilizes various agricultural wastes, forestry wastes and dairy, food and pulp and paper industries industry. Several reactor designs operating with solid state fermentation technology include packed bed reactor, fluidized bed reactor, rotating drum reactor, and stirred tank reactor, rocking drum reactor, stirred drum reactor and Zymotis reactor configurations. The SSF technology helps in management of solid and liquid wastes integrated with inexpensive biochemical production. The present paper includes practical data on lactic acid production by solid state fermentation technology utilizing wheat bran bed material and dairy waste whey and also highlights some of the technologies applicable for maximizing the biochemical production by SSF technology.

Keywords: Solid-state fermentation, fluidized bed, packed bed reactor, biochemical production.

Introduction

The twin environmental problems due to accumulation of large amount of agro industrial solid waste such as wheat bran, sugarcane bagasse, forestry wastes while liquid wastes emerging from dairy, food, pulp and paper industries industry pose a threat to water sources because of high BOD content can be solved by their utilization in solid state fermentation (SSF) technology. The SSF technology has the potential to generate value added chemicals and biomass by utilizing various agro residues and forestry wastes such as wheat bran, sugarcane bagasse, rice straw, pea peel waste, orange peel waste and pine needles have been applied as bed materials in production of biochemicals like lactic acid, xylitol, enzymes and amino acids[13]**.** Hence SSF technology integrates environmental cleanup with biochemical and biomass production. Large scale application of SSF technology involves the use of various bioreactor designs such as packed bed reactor, fluidized bed reactor, rotating drum reactor, and stirred tank reactor, rocking drum reactor, stirred drum reactor and Zymotis reactor configurations[4].

Lactic acid, a chiral carboxylic acid and a multifunctional compound that is applied as an acidulant, preservative and flavoring agent, probiotics and bacteriocins (through its microbes) in food and dairy industries and provides monomeric feedstock in production of ecofriendly biodegradable polymer (PLA) having biomedical uses. The world lactic acid production is predominantly through microbial fermentation rather than chemical synthesis which provides a mixture of isomers of lactic acid, whose separation is expensive[5]. Wheat bran constitutes a significant portion of agro-industrial waste produced in the flour mills. It forms the outer portion of the wheat seed, usually accounts for 14–19% of the grain. It comprises of the outer layers of grain, exocarp, mesocarp and endocarp (rich in minerals), the testa (rich in vitamins and enzymes), the aleurone layer (rich in protein and fats) and the remnants of the starchy endosperm[6]. Wheat is the second major food crop in India (about one third of the food grain production), with 26.6 million hectares of cropped land (2003-04) under it, having a productivity of 2,707kg/hectare while the global production of wheat was 585million tones (1999-2001) to which India contributed 12.3%[7]. It was reported by Sun et al. (2008), that, whole or hydrolyzed, wheat bran, enhanced the cellulase production in filamentous fungi and wheat bran consists of approximately about 19% starch, 18% crude protein, 58% non starch carbohydrates with 24% cellulose, 70% arabinoxylans and 6% glucans as major non starch polysaccharides and lignin[8]. Raw starch based media has been applied on wheat bran bed material for lactic acid production by amylolytic Lactobacilli[9]. Protease treated wheat bran, was utilized by mixed cultures of *L. delbrueckii* and *L. casei*, to produce lactic acid through simultaneous saccharification and fermentation[10]. Wheat bran could be utilized in solid state, liquid state fermentations and animal feeds. The sugars, nitrogenous substances such as proteins, amino acids and various vitamins (niacin, thiamine, riboflavin, vitamin A, vitamin K, vitamin E and folate etc.) and minerals(potassium, phosphorus, magnesium, calcium), in wheat bran, have the potential to support *Lactobacilli,* in growth and lactic acid production[11]. The bran part contains higher quantity of the amino acids (0.25g cysteine, 0.85g arginine, 0.54g lysine, 0.18g histidine and 0.28g tryptophan per 100g wheat bran) than the endosperm[12]. Some of the amino acids that are essential for growth and existence for lactobacilli are arginine, cysteine, tryptophane, histidine, tyrosine, isoleucine, glutamic acid,

valine, leucine, methionine, phenyl alanine, proline and threonine etc. Many of these amino acids can be supplied from the wheat bran bed material.

Several inexpensive waste materials generated in large amount that are rich in fermentable sugars, such as cheese whey and potato starch hydrolyzate, have been applied for solid state bacterial lactic acid production. Cheese whey has a BOD of 38,000 to 46,000 ppm and COD 80 g/L due to the presence of approximately 5% lactose, 0.06% fat, and 0.8 to 1% nitrogenous compounds[13]. About 10^8 tons per annum of cheese whey (containing approximately, 4.5-5% lactose, 0.8-1% protein, .06% fat, important vitamins and amino acids), produced as dairy by product, can be efficiently utilized as cheap carbon source for microbial preparation of lactic acid. Such bioconversion of whey to lactic acid can potentially bring down the cost of activated sludge treatment[14,15]. Lactose sugar contained in whey can be bio-converted into various fermentation products such as lactic acid, ethanol and single cell proteins, through fermentation, proved economical[16]. Solid state production process provides a suitable technology for utilization of solid agro-wastes as bed materials in synthesis and liberation of value added microbial fermentation products.

The present experiments were carried out with the following objectives to investigate i. The feasibility of solid state fermentation (SSF) in lactic acid production, utilizing a bed of wheat bran. ii. To observe the effectiveness of dairy whey as a substitute for expensive pure sugars in lactic acid production by SSF. Iii. To examine the effect of various doses of pure glucose or mixed with and whey as carbon source, on lactic acid production. iv. To determine the comparative acid production capabilities of the *Lactobacillus* pure strains and coculture in solid state fermentation.

Materials and Methods

Microbial cultures and media components: The chemicals used in these experiments were of Merck and High media make. Pure cultures of Lactobacilli (1) *L. delbrueckii* (NCIM2025) (2) *L. pentosus* (NCIM 2912) (3) *Lactobacillus sp.*(NCIM 2734) (4) *Lactobacillus sp.* (NCIM2084) had been acquired from National collection of industrial microorganisms (NCIM) of National Chemical Laboratory(NCL) Pune. The inoculum for the *Lactobacillus* strains were prepared in MRS (de Mann Rogosa Sharpe) media.

Composition of one liter MRS growth medium**:** 10 g proteose peptone, 5 g yeast extract, 10 g beef extract, 20 g dextrose, 1g Tween 80, 2 g ammonium citrate, 5 g sodium acetate, 0.1 g $MgSO_4.7H_2O$, 0.05 g $MnSO_4$, 2g K_2HPO_4 in distilled water as solvent.

Composition of one liter of synthetic production media: Carbon sources -Various levels of glucose (60, 80,110 and 120) g/L in pure production media and (30,40,65 and 70) g/L in whey substituted media(where the whey lactose provided rest of the 30,40,45 and 50) g/L to make the total sugar levels (60, 80,110 and 120) g/L again,1g sodium acetate, 0.03 g $MnSO_4.H_2O$, 0.10 g $MgSO_4.7H_2O$, 15 g yeast extract,0.25 g KH_2PO_4, 0.25 g K_2HPO_4 and 0.03 g $FeSO_4$. Sodium hydroxide at 2% level was applied as neutralizer.

Application of Production Media, Autoclaving, Inoculation and Incubation: This step was carried out aseptically in a laminar flow work station (Sanco make, Sandeep Instruments and Chemicals, New Delhi, India), which was disinfected with ethyl alcohol and irradiated with the ultraviolet lamp prior to use for one hour. Here 40mL of the production medium was added to each of the 250mL conical flasks containing 6g powdered bed of bagasse and subsequently autoclaved for sterilization, taking 15 minutes holding time at 15psi pressure. Then the flasks were left for cooling in the laminar flow cabinet under the ultraviolet light for one hour. Inoculums containing 2g/L of biomass of different strains of *Lactobacilli* were inoculated in different conical flasks, containing cool sterilized media in the bed and 1.0 mL of 2% NaOH neutralizer, and the flasks were tightly screw capped immediately. The incubator (Sanco make, Sandeep Instruments and Chemicals, New Delhi, India) was cleaned with disinfectants and the inner chamber was fumigated with a mixture of formalene solution and potassium permanganate, one day prior to incubation. The inoculated flasks were kept for incubation at 33 ^oC under static conditions in an incubator for six days. Carbon dioxide gas was supplied intermittently inside the closed incubator to maintain anaerobic conditions.

Preparation of bed material: The particle size fractions of the dried bed material were determined through a set of vibratory screens arranged vertically in order of decreasing mesh sizes, 1680, 1204, 710, 500, 420 and 150µ. Six grams of dried bran were weighed and uniformly dropped in each of 250 mL conical flasks.

Assays in Wheat Bran: Some of the important components in dried wheat bran such as pentosan and Klason lignin have been assayed by TAPPI method T223 cm-01, T222 om-06 and while starch and holocellulose were estimated by methods described by Holm[17,18]. Protein in the filtrates of autoclaved10% (w/v) wheat bran solution was estimated by Bradford method (1976) using bovine serum albumin as standard[19].

Bacterial Cell Concentration and Inoculum Preparation: The bacterial biomass was expressed as cell dry weight in gram per liter (g/L).Equal volumes of culture broth were filtered through pre-weighed microporous filter papers that were first washed with 0.85% sterile saline solution prepared in distilled water followed by drying at 80^0 C until attainment of constant weight. The biomass concentration in (g/L), is given by the difference of (initial and final weights of the filter paper) per volume of the broth passed through the filter paper (expressed in liter).The inoculum preparation for the *Lactobacillus* strains

under study was performed in standard MRS culture media. A loop full of pure culture obtained from the National Chemical Laboratory, Pune (India) were transferred from MRS agar stabs to the 50 mL MRS media contained in the Earlenmayer flasks , under aseptic conditions. In case of the coculture a loop full each from strain-1 and strain-2 were transferred to the MRS media. All the inoculated flasks were screw capped tightly and then kept in an incubator shaker at 33 ^{0}C, 180rpm for 14 hours. The biomass concentration of each culture was determined in form of cell dry weight.

Lactose estimation: Lactose content of the cheese whey was estimated by colorimetric method given by Nickerson et al.[20]. The absorbance of the sample was taken at 540 nm on a UV-VIS double beam Systronics 118, spectrophotometer.

Extraction and assay of lactic acid: After six days of incubation period, 50mL of distilled water was added to each conical flask and shaken for two hours at 200 rpm for better mixing and liberation of acid from the bed. Extraction of lactic acid was carried out by passing the mixture through a muslin cloth where the solid bed particles were removed and the pH of the extract collected was recorded with a digital pH meter.

The lactic acid present in the extracts were quantitatively assayed by the Kimberly - Taylor method[21]. The absorbance for lactic acid was measured in a UV-VIS double beam Systronics 118, India spectrophotometer at 570nm.

Scanning Electron Microscopy: Scanning electron microscopy (SEM) was performed using a LEO-435VP microscope to obtain clearly magnified view of the growth of *Lactobacillus* cells on the bed material. Very small portions of dried wheat bran beds (after inoculation and six days incubation except the control raw material), dried at 60 ^{o}C for 30 hours, were placed on the stubs and subjected to gold coating and observed at 15 kV.

FT-IR analysis: Fourier transform infrared spectroscopy (FT-IR) was performed using a Nicolet-6000 spectrophotometer to ascertain the presence of some of the functional groups associated with the bed material. Thoroughly washed (by distilled water) and dried wheat bran was used for FTIR. It was mixed with potassium bromide and pressed under vacuum. Transmittances were measured over a range near 500 to 4000 cm^{-1}.

Results and Discussion

Studies on the Ground Wheat Bran Bed Material: The particle size in the solid bed influences the production in solid state fermentation due to its relation with surface area, liquid holding capacity, gaseous exchange and mass transfer. Presence of a major portion of small sized particles in the bed provides larger surface area but have lesser mass transfer[22]. In this study the majority of wheat bran bed particles had size greater than 500µ, followed by particle sizes greater than 710µ, 420µ and 150µ.

Table-1
Composition of major constituents of dry wheat bran

Analyzed constituents of wheat bran	Weight %
Pentosan	31.02
Holocellulose	44.06
Klason Lignin	04.85
Starch	32.24
Crude Protein	14.62

Table-2
Particle size fractions of dried, wheat bran bed material obtained by sieving in a vibratory shaker through vertically arranged screens in order of diminishing pore sizes operated for 10 minutes

Mesh size (microns)	Weight% of the particles retained
1680	00.2288
1204	05.0691
710	24.5528
500	46.4736
420	14.5358
150	09.0984
Residual particles	00.2702

Lactic Acid Production on Wheat Bran Bed Material: The results in table 3 given below, revealed that maximum acid production were attained by all the *Lactobacillus* strains including the co-culture of the first two strains, at 80 g/L dose of pure glucose containing, synthetic production media. The results of lactic acid production at higher concentration of pure glucose treatment (120 g/L) in production media indicated that, the acid synthesizing ability of strain-1 and co-culture were reduced due to inhibition at high sugar concentration in comparison to their own maximum production values attained at 80 g/L glucose treatment, while a significant decline in the lactic acid production of strain 2, 3 and 4 were observed, indicating their higher susceptibility to inhibition due to higher sugar concentration. Compared to all the strains under different levels of glucose treatments, the co-culture in table one shows the maximum lactic acid production 50.12 g/L (mean value) at a glucose level of 80 g/L) closely followed by strain-1, 46.10 g/L . The co-culture showed better acid production (lower pH and higher lactic yield) than its constituent strains (1) and (2) with first two doses of glucose in table 3, while it drops) at 120 g/L dose of pure glucose, which highlights the fact that amongst all strains, the strain-1 is best suitable (against inhibition) for the lactic acid production at high concentration of glucose. The strain-2 *L. pentosus* has lower concentration of lactic acid since it is primarily pentose sugar utilizer but the supply of glucose, a C-6 carbon source, may have reduced the lactic acid production by it.

In table 4 the coculture again exhibited highest lactic acid production, 50.12 g/L in comparison to other strains and its constituent strains (1) and (2) in table 4 at 120 g/L dose of whey substituted glucose. In table 4 all the strains followed gradually increasing trend of lactic acid production with the increase in the levels of glucose and whey substitution. All the strains attained their highest acid yields with the highest level of sugar (120 g/L) in table 4, while the same sugar level in table 3, indicated an inhibition, for all these *Lactobacilli* strains. All the pure strains and coculture of lactobacilli under study exhibited good compatibility with the whey substituted glucose, as evident from the highest values of lactic acid concentration in table 4, that were marginally lesser than their maximum values observed in table 3 (in pure glucose). This observation may be probably due to several possible reasons such as lesser inhibition of their lac operon by glucose, presence of other lactose uptake systems, capability to utilize pentose sugars (due to hemicellulose degradation under high pressure, temperature and acidic conditions while autoclaving)or higher cell numbers due to stimulatory substances from whey.

Table-3
Lactic Acid Production obtained in wheat bran bed material, from the different strains of *lactobacilli* in corresponding to various doses of pure glucose applied in the production media

Bacterial strains	Glucose 60g/L		Glucose 80g/L		Glucose 110g/L		Glucose 120g/L	
	pH	LA (g/L)*	pH	LA (g/L)*	pH	LA (g/L)*	pH	LA (g/L)*
L. delbrueckii NCIM2025 (Strain-1)	4.10 ±0.011	37.12±0.60	3.85 ±0.012	46.10±0.61	3.89 ±0.019	41.20±0.54	3.96 ±0.026	37.0±0.50
L. pentosus NCIM2912 (Strain-2)	4.75 ±0.028	8.51±0.19	4.60 ±0.049	22.15±0.36	4.65 ±0.056	20.05±0.41	5.94 ±0.038	12.11±0.23
Coculture of first two strains	3.89 ±0.014	35.10±0.21	3.82 ±0.010	50.12±0.54	3.91 ±0.011	43.91±0.50	3.98 ±0.029	39.00±0.37
Lactobacillus sp. NCIM2734 (Strain-3)	4.81 ±0. 050	13.2±0.16	4.75 ±0.044	22.50±0.41	4.81 ±0.031	10.14±0.43	5.78 ±0.044	8.64±0.35
Lactobacillus sp. NCIM2084 (Strain-4)	5.40 ±0.046	9.21±0.21	4.78 ±0.039	26.61±0.68	4.87 ±0.017	19.15±0.53	5.98 ±0.060	5.01±0.14

LA- Lactic acid;* in extract; (Results given as mean ± standard deviation, based on repeated trials) at 33^0C.

Table-4
Lactic Acid Production obtained in Wheat Bran Bed Material by Different Strains of *Lactobacilli* Corresponding to Various Doses of Whey Lactose And Glucose In Production Medium

Bacterial strains	**Glucose 30g/ L +WL30g/ L TS 60g/ L**		**Glucose 40g/ L +WL40g/ L TS 80g/ L**		**Glucose 65g/ L +WL45g/ L TS 110g/ L**		**Glucose 70g/ L +WL50g/ L TS 120g/ L**	
	pH	**LA (g/L)***	**pH**	**LA (g/L)***	**pH**	**LA (g/L)***	**pH**	**LA (g/L)***
L. delbrueckii NCIM2025 (Strain-1)	4.20±0.021	35.12±0.50	4.05±0.035	37.55±0.40	3.95±0.023	40.06±0.49	3.82±0.019	43.85±0.48
L. pentosus NCIM2912 (Strain-2)	5.88±0.038	6.50±0.16	5.08±0.070	12.18±0.26	4.78±0.060	17.31±0.21	4.61±0.031	21.65±0.46
Coculture of first two strains	4.36±0.052	24.30±0.37	3.88±0.031	41.00±0.49	3.70±0.018	45.03±0.51	3.62±0.011	50.12±0.48
Lactobacillus sp. NCIM2734 (Strain-3)	5.39±0.061	13.10±0.28	4.90±0.065	15.75±0.38	4.58±0.048	20.01±0.38	4.47±0.041	23.16±0.43
Lactobacillus sp. NCIM2084 (Strain-4)	5.48±0.066	8.00±0.12	4.59±0.044	17.57±0.38	4.50±0.046	20.27±0.34	4.32±0.037	26.03±0.57

WL-Whey lactose; TS-Total sugar; LA- Lactic acid;* in extract (Results given as mean ± standard deviation, based on repeated trials) at 33^0C.

A closer comparison between table 3 and table 4, showed that the co-culture attained a maximum production of 50.12g/L lactic acid at 80 g/L pure glucose level, while the same lactic acid concentration (50.12 g/L) had been achieved with 120 g/L whey substituted glucose.

This signifies that the co-culture could efficiently utilize higher doses of whey substituted glucose, without any significant inhibition, and provide highest lactic acid production that equals with the production level obtained with pure sugar glucose as sole carbon source. Hence coculture has better performance in lactic acid production industries based on the utilization of inexpensive carbon sources such as whey.

Changes in Bed Material under Autoclaving Conditions: The co-culture displayed the capacity of utilizing whey lactose and glucose sugar and pentose sugars (which might have possibly liberated from the pentosan fraction of bed material 31.02% during autoclaving as per table-1) while the pure strain-1 (which was one of the constituent strains in co-culture), has not been known to utilize the pentose sugars. The high temperature (100-120^{o}C), pressure and slightly acidic pH conditions, which prevailed during autoclaving, might have possibly degraded the acetylated xylans in the bed material to xylan and acetic acid[23]. Autohydrolysis or dilute acid hydrolysis reactions possible during autoclaving might have lead to the degradation of hemicellulose. The hydronium ions required for both the processes, may be either present before or emerged later through the auto-ionization of water, and subsequently depolymerized hemicelluloses, by hydrolysis of glycosidic linkages and acetyl groups. The autohydrolysis has been reported to convert hemicelluloses into soluble monosaccharides or oligosaccharides, with reduced amount of byproducts[24]. Presence of 2% sodium hydroxide neutralizer in the bed could have enhanced the degradation of lignin polymer in the wheat bran bed material.

The cheese whey, contains several proteins, of which major proteins are α- lactoglobulin, β-lactoglobulin and serum albumin while minor proteins consist of glycomacro peptide (GMP), lactoferrin, bovine serum albumin, immunoglobulin, lactoperoxidase, peptones and phospho-lipoprotein[25]. These proteins can provide important source of amino acids along with the proteins from wheat bran to inoculated lactic acid bacteria after their probable heat denaturation during autoclaving. Thus the *Lactobacillus* strains which are inoculated after autoclaving and bringing the temperature at about 30^{o} C can obtain amino acids from the bed material which shall help in growth and production of lactic acid.

The lactic acid production of all the strains in table 4 maintained an increasing trend with enhancement in dose of whey in production media. This observation was probably due to the role of growth activating substances from whey and contributions of nitrogenous substances(amino acids) from wheat bran, which resulted into enhanced biomass growth, responsible for faster utilization of glucose (the repressor of lac operon). The rapid removal of glucose helps to restore the activity of lac operon and indirectly help faster lactose uptake. The increasing doses of lactose, which is a well known inducer for the lactose operon, plays an active role in activation of the lac operon, through inactivation of the repressor protein molecules by conformational change, induced due to its binding with glucose repressor. Highest mean lactic acid concentrations of 50.12g/L and 43.85g/L achieved by coculture and strain-1 at 120 g/L sugar dose) in table 4.

1(a) **1(b)** **1(c)** **1(d)** **1(e)**

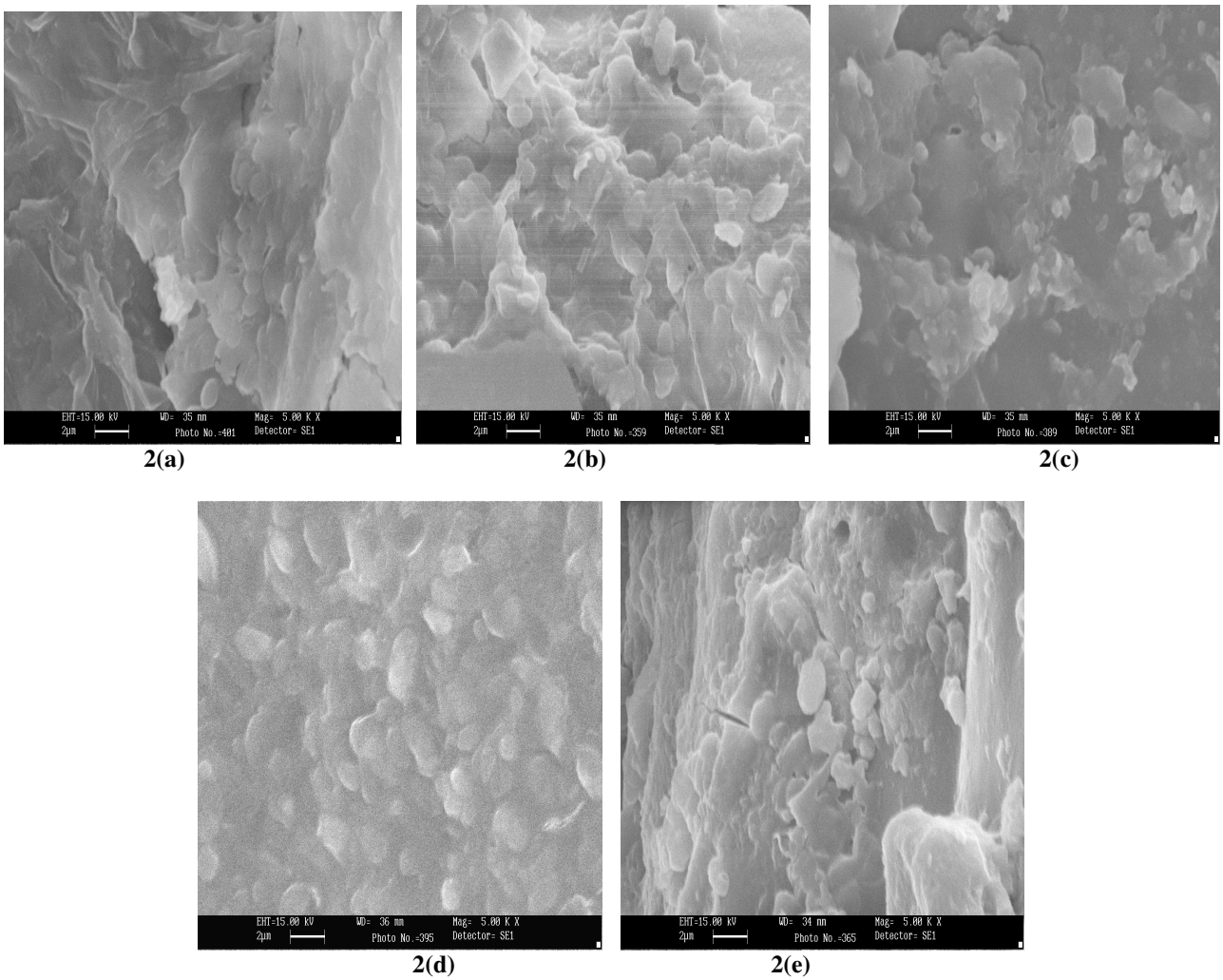
Figure-1
Growth of *Lactobacilli* on wheat bran bed material

Frames 1(a), 1(b), 1(c), 1(d) and 1(e) show growth of strains-1,2, 3,4 and coculture on 120g/L whey substituted glucose media.

The frames 1(a), 1(b), 1(c, 1(d) and 1(e) depict the growth of lactic acid yielding pure strains 1,2,3,4 and coculture, utilizing whey substituted glucose at 120g/L level as carbon source.

Scanning Electron Micrographs of Bacterial Growth on the bed of Wheat Bran

Groups of Lactobacilli cells of strains-1,2, 3, 4, and co-coculture are observed in the SEM micrographs Figure-2: 2(a),2(b), 2(c), 2(d), and 2(e), as fused growth, distinct capsular rod-shaped bacterial cells or seen to form aggregates adhering with the bed material at 120 g/L whey-substituted glucose media application (under 5000X magnification). Figure 2(e) for co-culture indicated isolated capsular cells and also fused growth of bacteria very closely aggregated to each other provided the highest lactic acid production of 50.12 g/L mean value (mentioned in table 4). The aggregates as well as separate rod shaped Lactobacilli cells adhering to the bed material can be observed in the SEM micrographs in Figure-2. The better growth clearly indicates that the present bacterial strains bear the capacity to grow on the bed material utilizing stimulatory substances from bed materials as well as whey, despite the possibility of the release of some inhibitory phenolic substances from the bed material during autoclaving.



2(a) **2(b)** **2(c)**

2(d) **2(e)**

Figure-2

SEM micrographs show the growth of Lactobacilli on solid bed of wheat bran. Micrographs 2(a), 2(b), 2(c), 2(d), and 2(e) show the growth of the strains-1, 2, 3, 4, and coculture, respectively, at 5000X magnification, on wheat bran bed fed with a production media containing whey lactose (50 g/L), combined with glucose (70 g/L) at 120 g/L total sugar level, after six days incubation at 33^0C

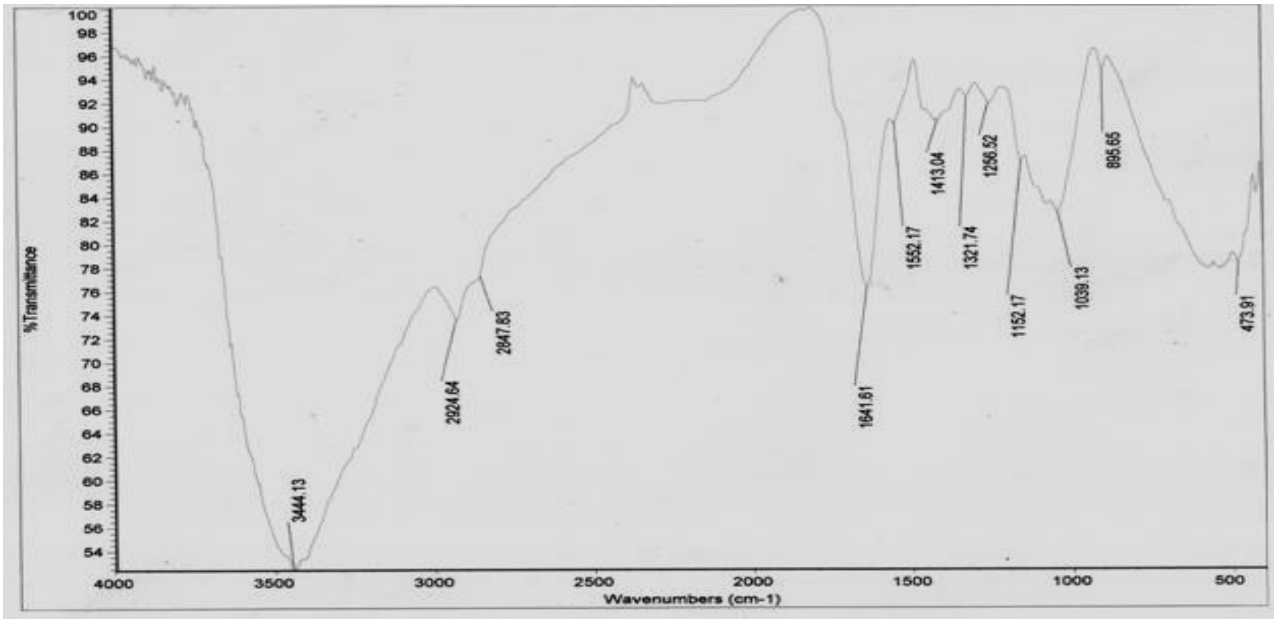


Figure-3
FT-IR spectra of a wheat bran bed sample in dried state

FTIR Studies of Wheat Bran: The FT-IR analysis of dried wheat bran, as per figure-3, indicated the peaks corresponding different functional groups associated with various compounds predicted in the wheat bran bed material. The FT-IR studies of the bed materials after autoclaving are important for prediction of compounds that could be liberated by degradation reactions, under high temperature, pressure and mild acidic conditions, prevailing during autoclaving, hence might have affected the lactic acid producing bacterial strains under study. The peak near 1152 cm^{-1} could be attributed to C-O, C-O-C stretching, C-OH bending vibrations in arabinoxylans and also indicated glycosidic linkages[26]. The absorption peak around 1039 indicated C-N stretching of amines and –CO stretching of carboxylic acid group –COOH 27.The band located at 1256 cm^{-1} showed OH deformations of carboxylic acid. The peak located around 1641 cm^{-1} represented the –C=O stretching of hydroxyl, aldehyde or ketones with hydrogen bonding,-C=C stretching and –C=N stretching 27. The peak at 1552 cm^{-1} results due to amide stretching and bending vibrations of proteins[28]. The spectral region near peak observed at 3444cm^{-1} suggested, O-H stretching vibrations in guaiacyl and syringyl rings(which form monomer units in lignin) under intramolecular hydrogen bonding while the peak near 1321 cm^{-1} may be attributed due to C-O stretching of phenol[26]. The peak detected at 2924cm^{-1} indicated symmetric C-H stretching vibration and –OH stretching of carboxylic group[27].

Conclusion

Wheat bran, a cheap agro industrial waste, is available from the flour mills in large quantities can be a potential bed material for supporting the growth and acid production of the *Lactobacillus* strains. The coculture or mixed culture shows higher acid production than its constituent strains provide individually and also higher in comparison to other strains of *Lactobacilli* in both, pure glucose or whey substituted glucose based media. The coculture showed better performance with higher doses of whey, hence it can be suitable in fermentation industries for lactic acid production. Highest lactic acid production values for all the strains, occurred at 80 g/L dose of pure glucose and 120 g/L of whey substituted glucose media. Utilization of SSF technology on wheat bran solid waste (containing important sugar and nitrogen components such as proteins and amino acids) with dairy whey lactose partially substituting pure glucose for production of lactic acid provides a potentially sound technology for valuable biochemical production from abundant liquid and solid wastes from dairy and agro industries.

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