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Original Article

Potential of cellulolytic bacteria for ethanol production from agri-food wastes

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Abstract

Bacterial isolates were cultivated in aqueous extracts of watermelon's rinds, its pulp, peels of mangoes and apples and sugarcane bagasse. The isolate designated as AI-1 showed maximum growth with initial pH 5 at 30°C with 10% of 72 hours old inoculums. The isolate AI-2 and AI-3 grew best at 45 C and 37 C with initial pH 9 and 10% 72 hours old inoculum. The isolate AI-4 showed maximum growth at 45°C and initial pH 9 and 10% 72 hours old inoculum. The bacterial isolates AI-5 and AI-6 showed maximum growth at 30 C with initial pH 9 with 5% and 10% 48 and 24 hours old of inocula size and age, respectively. The isolates AI-2, AI-4, AI-5 and AI-6 were found to be ethanologenic when cultured in batch fermentation for five days. The bacterium AI-6 grew best in aqueous extract of watermelon rinds and sugarcane bagasse and yielded up to 7.4% ethanol at 6th day of fermentation. The reported bacterial diversity can be exploited for conversion of wastes into ethanol and bacterial cell biomass under a range of different physicochemical parameters *i.e,* pH and temperature. Neutral pH and higher temperature optima for these isolates make them good candidates for obtaining bioethanol from agriwastes in non-aseptic conditions.

Key words: Bioethanol, sugarcane bagasse, watermelons, batch fermentation

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INTRODUCTION

With growing human population and industrialization, the fossil fuels energy resources are not only declining but also add, obnoxious oxides of nitrogen and sulfur, which in turn deteriorate our environment (Quadrelli and Peterson, 2007). The present energy status of fossil fuels turns the scientist towards searching renewable and environmentally safe energy resources (Akpmar *et al*., 2008; Dong *et al*. 2008; Deenanath *et al*., 2012; Sarkar *et al.,* 2012). Biofuels may decrease dependency on fossil fuel utilization, and reduce the greenhouse gases in near future. The biofuels include bioethanol, biobutanol, biodiesel, vegetable oils, biomethanol, pyrolysis oils, biogas, and biohydrogen. The use of gasoline and diesels may be replaced by bioethanol and biodiesel, respectively (Demirbas, 2005; Demirbas and Dincer, 2009). Primary feedstocks of bioethanol are lignocellulosic materials such as sugarcane and corn (Chaudhary and Qazi, 2008; Shakar *et al*., 2012). About 60% of global bioethanol production comes from sugarcane and 40% from other crops. Bioethanol is environmentally safe and clean substitutive of gasoline or is mixed with gasoline. Gasohol the mixture of gasoline and ethanol is an alternative fuel for vehicles. Ethanol contains 35% of oxygen, so its burning minimizes the emission of CO_2 (Demirbas and Dincer, 2009; Deenanath *et al*., 2012; Sarkar *et al*., 2012).

Agro industrial sector has shown tremendrous development since last few decades. Consequently, accumulation of large amounts of lignocellulosic residues around the world has started to increase. Currently in Brazil, 59% of 273×10^{6} tons of sugarcane is employed for ethanol productions while the rest for sugar production. This implies a huge amount of sugarcane bagasse waste generation (Soccol *et al*., 2010; Deenanath *et al*., 2012). Microbial conversion of such wastes into useful and value added products seems to be a promising method for production of biofuel and solid waste management (Sun and Cheng 2002; Sukumaran *et al*., 2005; Chaudhary and Qazi, 2008; Balat,

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2011). Thus identification and process development of suitable wastes is necessary for economically feasible and sustainable supplies of bioenergy. The present study aimed conversion of low cost agro industrial wastes into bioethanol employing cellulolytic and ethanologenic bacteria.

MATERIALS AND METHODS

Collection and processing of the substrates

Wastes of water melons rind, mangoes and apple's peels and sugarcanes bagasse were collected from local fruit shops of the Lahore. Watermelons were purchased for collection of pulp. Rind of watermelons, mangoes and apple's peels, watermelon pulp and sugarcane bagasse were washed, air dried in sunlight and then kept in an oven at 80 $^{\circ}$C for 1 to 2 weeks till consistent weight was achieved. After drying, the substrates were ground and stored properly.

Biochemical identification of isolates

The already isolated bacterial strains AI-1, AI-2, AI-3, AI-4, AI-5 and AI-6 present in stock depository of Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab (Quaid-e-Azam campus), Lahore were revived in nutrient broth. The broth cultures were employed for determination of Gram's reaction, motility (hanging drop method), endospore, and oxidase and catalase activities according to the procedures described by Benson (1994).

Estimation of cellulose production potential of select isolates

Cellulase selective agar medium was prepared according to the composition described by Ogbonna *et al*. (1994) with slight modification made by Saeed (2005). Then bacterial isolates were inoculated on the plates. After 24 hours of incubation at 37 ºC freshly prepared Gram's iodine solution was added on the plates. Appearing of the clear zones around bacterial colonies indicated clearance of cellulose from that region due to the production of cellulase exoenzymes (Kasana, *et al*., 2008). Then cellulase enzyme units were determined for the studied strains by O' toludine method according to Hartel *et al*. (1969).

Selection of the media for isolated strains

Revived strains of cellulolytic bacteria were cultivated in 0.2% aqueous extract of apple (A), bagasse (B), mango (C), watermelon (D), separately and in their different combinations. Best growth media for each of six strains were selected by recording optical density (OD) at 600 nm and were further optimized for temperature (30, 37 and 45 °C), oxygen (aerobic, anaerobic natures), pH (5, 7 and 9.0) and inoculum size (1, 5 and 10%) and Inoculum age (24, 48 and 72 hours).

Effect of watermelon pulp on isolates' growth

To determine the effect of addition of watermelon pulp on the growth of respective isolates in their respective optimized growth conditions, 0.2 % aqueous extracts were prepared for each respective bacterial isolate with and without addition of 0.1 % water melon pulp. After 24 hours of incubation O.D were recorded at 600 nm.

Alcohol estimation produced by selective strains

Each respective medium (50 ml) was taken into culture bottles, autoclaved and inoculated bacterial isolates their respective optimized growth conditions for 5 days. After five days fermentation of the substrate with pre-grown bacterial mass was covered with autoclaved paraffin oil for anaerobic condition. Alcohol was estimated for each of the bacterial strains by modifying the method of Snell and Snell (1973).

Biochemical analysis of media substrate

Total carbohydrates and soluble protein contents of the media substrates were estimated before and after fermentation by phenol sulphuric acid method (Dubios *et al*., 1956) and Folin-Ciocalteu method (Lowry *et al*., 1951), respectively.

Batch fermentation in 125 ml culture bottles

Isolate AI-6 was selected for further study in batch fermentation in 125 ml culture bottles under optimized growth conditions. One hundred ml of medium (bagasse+watermelon) were taken in culture bottle, autoclaved and inoculated with isolate AI-6 and incubated at its respective growth optima for 5 days. After five days' fermentation of the substrate with pre-grown bacterial mass fresh media were added and culture bottles were air tightened to create anaerobic conditions. Daily sample was taken to study the parameters like strain growth, carbohydrate and proteins content and ethanol production.

Batch fermentation in 1.5 L Fermenter

The optimized growth conditions were then further scaled up to fermenter level (Fig. 1). One litre of medium (Bagasse+Watermelon) was taken into fermenter of 1.5 Liter capacity and proceeded as described above.

Figure 1 Fermentation of the isolate AI-6 in fermenter of 1.5 Liters.

Ethanol Estimation

Ethanol contents of cultured media both from culture bottles and fermenter were estimated the method of Snell and Snell (1973) with slight modification made by Saeed (2005).

RESULTS

Biochemical characterization and cellulose activity of the isolates

In present study, all bacterial isolates were found Gram positive, catalase positive, oxidase positive, endospore former and rod shaped. The isolates AI-1, AI-2 and AI-4 appeared slime formers. The isolates AI-1 to AI-5 formed oval shaped endospores. While the isolate AI-6 had cylindrical and central in position endosperes. Isolates AI-1, AI-2, AI-5 and AI-6 were single celled (Table I).

All the bacterial isolates appeared cellulolytic when grown on selective cellulose medium. Clear zones of cellulose hydrolysis appeared on plates of cellulose medium and the isolates AI-1, AI-2, AI-3, AI-4, AI-5, and AI-6 yielded the zones up to 0.8±0.07, 0.95±0.18, 0.37±0.03, 1.00±0.028, 0.4±0.057 and 1.01±0.04mm, respectively. All the bacterial isolates; AI-1, AI-2, AI-3, AI-4, AI-5, and AI-6 yielded upto 0.72±0.001, 1.927±0.003, 0.888±0.01, 0.92±0.002, 0.371±0.003 and 0.98±0.001 units of cellulase respectively following 24hours of incubation in broth of cellulose medium (Table I).

Cultivation of the isolates in different aqueous extracts of fruits wastes

Cultivation of the bacterial strains in different media comprising of fruit wastes indicated that the isolates had a potential of utilizing peels of apple and mango, rind and pulp of watermelon and bagasse. The bacterial isolate AI-1 yielded highest growth in bagasse and watermelon rinds' extract and attained O.D of 0.325±0.026 after 24 hours at 37°C. While the bacterial isolate AI-6 yielded highest growth in Bagasse and Watermelon peels' extract and could attain O.D value of 0.201±0.018 in 24 hours at 37^{0}C (Table II).

Optimization of growth conditions of the bacterial isolates

At 24 hours post incubation, the bacterial strains AI-1, AI-5 and AI-6 showed best growth at 30^{0}C and the cells densities reached up to 0.147±0.021, 0.222±0.009 and 0.316±0.028, respectively. The bacteria AI-2 and AI-4 yielded best growth at 45^{0}C and O.D reached up to 0.216±0.003 and 0.158±0.012 respectively while the isolate AI-3 showed best growth at 37^{0}C and O.D reached up to 0.177±0.011. At 24 hours post incubation, the bacterial strain AI-1 showed best growth at initial pH 5 and O.D reached up to 0.251±0.005. While the isolates AI-2, AI-3, AI-4 and AI-5 yielded best growth at initial pH 9 and O.D reached up to 0.326±0.013, 0.352±0.002, 0.316±0.018 and 0.305±0.017 respectively after 24 hours of incubation at their respective optimized

temperatures. Isolate AI-6 grew best at pH 6 and O.D reached up to 0.150 ± 0.002.

Under the respective optimum growth conditions of temperature and pH after 24 hours of post incubation, the isolates AI-1 and AI-4 grew best at aeration and O.D reached up to 0.498±0.077 and 0.164±0.041, respectively. While the isolates AI-2, AI-3, AI-5 and AI-6 showed highest growth without aeration and O.D reached up to 0.126±0.012, 0.216±0.051, 0.242±0.016 and 0.129±0.0213, respectively.

Under the respective optimum conditions of temperature, pH and aeration after 24 hours of post incubation the isolates AI-1, AI-2, AI-3, AI-4 and AI-6 yielded best growth with inoculum size of 10% and the cells densities reached up to 0.319 ± 0.11, 0.273 ± 0.015, 0.344 ± 0.009, 0.264 ± 0.011 and 0.264 ± 0.024, respectively. While AI-5 grew best with inoculum size of 5% with O.D of 0.368 ± 0.006. Under the respective optimum growth conditions of temperature, pH and aeration and inocula age of 24, 48 and 72 hours were tried. The isolates AI-1, AI-2, AI-3 and AI-4 grew best with inoculum age of 72 hours with corresponding O.D of 0.260±0.020, 0.373±0.015, 0.457±0.021 and 0.590±0.026 respectively after 24 hours of incubation. The isolate AI-5 showed best growth with inoculum age of 48 hours and the O.D reached up to 0.247±0.020. While the isolate AI-6 yielded best growth with inoculum age 24 hours with O.D density of 0.247 ± 0.023.

Table I: Characterization of the bacterial isolates

Characteristics	Isolate code					
	AI-1	AI-2	AI-3	AI-4	AI-5	AI-6
Oxidase	Positive	Positive	Positive	Positive	weakly Positive	Weakly Positive
Gram staining	Positive	Positive	Positive	Positive	Positive	Positive
Endospore	Positive, oval and central	Positive, oval and central	Positive, oval and central	Positive, oval and central	Positive, oval and central	Cylindrical and central in position
Motility	Motile	Motile	Motile	Motile	Motile	Motile
Cellulose hydrolysis zones (mm)	0.8±0.07	0.95±0.18	0.37±0.03	1±0.028	0.4±0.057	1.01±0.04
Cellulase Enzyme units*	0.72±0.001	1.927±0.003	0.888±0.01	0.92±0.002	0.371±0.003	0.98±0.001

*One unit of cellulose is the amount of enzyme which released one mole of glucose/minute under assay condition.

Table II: Cultivation of the bacterial isolates in aqueous extract of 0.2% fruits wastes' and their combinations for preliminary screening of the substrates

Medium Ingredient(s)	Bacterial Isolates						
	AI-1	AI-2	AI-3	AI-4	AI-5	AI-6	Sig. Level
A	0.23^{b}±0.003	0.12^{c}±0.002	**0.31^{a}±0.017**	0.10^{cd}±0.003	0.05^{d}±0.016	0.14^{c}±0.009	0.000
B	0.11^{ab}±0.007	0.13^{a}±0.007	0.16^{a}±0.002	0.07^{b}±0.017	0.12^{ab}±0.011	0.11^{ab}±0.014	0.002
C	0.05^{bc}±0.013	0.13^{a}±0.016	0.08^{abc}±0.006	0.033^{c}±0.021	0.10^{ab}±0.002	0.10^{ab}±0.005	0.001
D	0.20^{bc}±0.003	**0.22^{b}±0.011**	0.21^{bc}± 0.016	0.15^{cd}±0.003	0.33^{a}±0.008	0.137^{d}±0.019	0.000
A+B (1:1)	0.21^{a}± 0.004	0.04^{cd}±0.011	0.12^{bc}±0.031	0.02^{d}±0.010	0.13^{ab}±0.027	0.05^{bcd}±0.014	0.000
A+C (1:1)	0.11^{b}±0.005	0.11^{b}±0.001	0.14^{a}± 0.001	0.03^{d}±0.003	0.04^{d}±0.006	0.08^{c}±0.007	0.000
A+D (1:1)	0.14^{ab}±0.016	0.12^{ab}±0.001	0.07^{ab}±0.044	0.02^{b}±0.007	0.14^{ab}±0.053	0.19^{a}±0.005	0.021
B+C (1:1)	**0.32^{a}± 0.026**	0.02^{cd}±0.007	0^{d}± 0.00	0.08^{bc}±0.002	0.15^{b}±0.027	0.12^{b}±0.006	0.000

B+D (1:1)	0.13^{cd}±0.00 1	0.11^{d}± 0.000	0.14^{cd}±0.003	**0.17^{bc}±0.009**	**0.47^{a}±0.009**	**0.20^{b}±0.018**	0.000
A+B+C+D	0.20^{b}± 0.001	0.09^{cd}±0.003	0.19^{b}± 0.041	0.16^{bc}±0.003	0.47^{a}±0.004	0.07^{d}±0.002	0.000

Apple peels =A; Bagasse peels = B, Mangoes peels = C and Watermelon rind = D; values are mean±SEM of three replicates that represent O.D of the bacterial cultures at 600nm of 24 hours incubated cultures.
Mean with the same letter in row and column did not differ significantly. The effects were declared highly significant if p<0.001, very significant if p<0.01 and significant if p<0.05

Under the respective optimized growth conditions of temperature, pH, aeration, inoculum size and age, the bacterial isolates were grown in the presence of 0.1% watermelon pulp. The isolates AI-1, AI-2, AI-3, AI-5 and AI-6 showed highest growth and the O.D reached up to 0.161± 0.005, 0.566± 0.043, 0.189± 0.011, 0.217± 0.015 and 0.265± 0.012, respectively at 24 hours of incubation, whereas the Isolate AI-4 yielded best growth in the absence of pulp with O.D value of 0.132± 0.013. The isolates AI-2, AI-5 and AI-6 appeared ethanologenic. The isolates AI-1 to AI-6 grew up to 0.109±0.006, 0.115±0.008, 0.076±0.008, 0.151±0.004, 0.133±0.01 and 0.287±0.002 O.D in B+C, D, A, B+D, B+D and B+D and yielded 0.77±0.348, 2.99±0.043, 0±0, 3.31±0.248, 2.55±0.102 and 4.71±0.066% ethanol respectively. Isolate AI-6 gave highest yield of 4.71±0.066% (Tables III).

Table III: Ethanol production by the bacterial isolates following cultivation their respective optimized media and incubation conditions at 5th day of the inoculation

Isolate Code	Substrate	Ethanol percentage v/v	O.D at 600nm
AI-1	B+C	0.77±0.348	0.109^{cd}±0.006
AI-2	D	2.99±0.043	0.115^{bcd}±0.008
AI-3	A	0±0	0.076^{d}±0.008
AI-4	B+D	3.31±0.248	0.151^{bc}±0.004
AI-5	B+D	2.55±0.102	0.133^{b}±0.01
AI-6	B+D	4.71±0.066	0.287^{a}±0.002

Values are mean±S.E.M of three replicates that represent O.D of the bacterial cultures at 600nm of 5 days incubated cultures and ethanol %v/v. Apple peels =A; Bagasse peels = B; Mango peels = C and Watermelon rind = D
Mean with the same letter in column did not differ significantly. The effect were declared highly significant if p<0.001, very significant if p<0.01 and significant if p<0.05

Table IV: Growth Kinetics of Isolate AI-6 in B+D substrates at various hrs. post incubation

Incubation Hours	Cultivation	Carbohydrate (µg/ml)	Protein (µg/ml)	Culture O.D	Ethanol percent (v/v)
0	Fermenter	59.80^{a}±11.49	170^{ab}±3.23	0.108^{ab}±0.002	0.02±0.029
	Batch	156.7^{a}±1.49	135.68^{a}±1.29	0.150^{b}±0.002	0.00±0.00
24	Fermenter	50.46^{ab}±2.30	182.15^{ab}±12.96	0.121^{a}±0.006	3.27±0.371
	Batch	134.08^{b}±8.24	126.73^{a}±2.51	0.193^{a}±0.015	3.17±0.118
48	Fermenter	41.3^{abc}±7.26	223.62^{a}±18.15	0.112^{a}±0.007	4.4±0.473
	Batch	116.63^{b}±0.663	119.28^{abc}±3.78	0.209^{a}±0.010	3.63±0.100
72	Fermenter	28.08^{bc}±1.49	172.523^{ab}±6.91	0.11^{ab}±0.009	5.43±0.115
	Batch	70.59^{c}±2.784	107.10^{bcd}±2.36	0.216^{a}±0.004	4.17±0.209
96	Fermenter	24.15^{bc}±0.14	172^{ab}±14.86	0.097^{ab}±0.004	7.42±0.259
	Batch	47.92^{d}±5.078	127.23^{a}±4.73	0.216^{a}±0.005	6.02±0.103
144	Fermenter	19.55^{c}±0.92	158.04^{bc}±7.39	0.087^{ab}±0.017	7.08±0.112

	Batch	11.53^{e}±3.42	103.12^{cd}±7.07	0.112^{bc}±0.007	7.35±0.052
168	Fermenter	16.40^{c}±0.51	106.35^{c}±15.88	0.069^{b}±0.001	7.13±0.069
	Batch	8.88^{e}±1.49	94.17^{d}±1.41	0.106^{d}±0.005	6.77±0.119
Significance Level (P)		<0.001	<0.001	<0.01	<0.001

For details of statistical analysis and values, see Table I.

Carbohydrates and proteins contents of aqueous substrates

Concentration of total soluble carbohydrates and proteins contents of uninoculated aqueous extracts of the agro industrial wastes had varying concentrations. Soluble carbohydrates and proteins contents of watermelon aqueous extract were 106.58±0.62µg/ml and 94.67±6.07µg/ml, respectively in uninoculated media but after addition of 0.1% watermelons pulp total parameters increased up to 133.43±4.76µg/ml and 131.28±4.16µg/ml. In other words carbohydrates and proteins contents after addition of pulp increased up to 25% and 38.6%, respectively. Total soluble carbohydrates and proteins contents of sugarcane bagasse aqueous extract were 109.02±1.77µg/ml and 53.91±2.24µg/ml, respectively in uninoculated media but after addition of 0.1% watermelons pulp total parameters increased up to 117.12±6.05µg/ml and 96±9.004µg/ml respectively. In other words carbohydrates and proteins contents after addition of pulp increased upto 7.4% and 78% respectively (Table IV).

Growth Kinetics of the Isolate AI-6

After inoculation of the isolate AI-6 in B+D+P, growth were measured as 0.150±0.002, 0.193±0.015, 0.209±0.010, 0.216±0.004, 0.216±0.005, 0.212±0.006, 0.112±0.007 and 0.106±0.005 O.D at 0, 24, 48, 72, 96, 120, 144 and 168 hours of incubation, respectively. Ethanol concentration increased after regular interval of 24 hours with 3.17±0.118, 3.63±0.100, 4.17±0.209, 6.02±0.103, 6.88±0.124, and 7.35±0.052 and 6.77±0.119% v/v. Ethanol production increased up to 144 hrs. and then decreased. Highest ethanol concentration after 144 hrs. of fermentation of the substrate was found approximate 7.35±0.052%v/v (Table IV). On the other hand, following inoculation of the isolate AI-6 in 1.5 liter capacity fermenter, carbohydrates decreased down to 63, 69, 74, 82, 85, 88, and 90% while the corresponding change in Proteins contents were 37%, 33%, 18%, 37%, 37%, 42%, and 60% respectively after 0, 24, 48, 72, 96, 144 and 168 hrs of fermentation, respectively. Growth increased up to 72 hours and then decreased. Ethanol concentration increased after regular interval of 24 hours with 0.02±0.029, 3.27±0.371, 4.4±0.473, 5.43±0.115, 7.42±0.259, 7.08±0.112 and 7.13±0.069%v/v. Ethanol production increased up to 96 hours and then decreased. Highest ethanol concentration after 96 hours of fermentation of the substrate found 7.42±0.259%v/v. (Table IV).

DISCUSSION

In the summer season watermelons crops are harvested and are available in bulk in big cities. Whereas due to low price and hence low levels of storage facilities tons of watermelons get wasted. Microbial decomposition often soften them and insects including house flies and honey bees are commonly observed navigating them. There is huge production of edible fruits in tropical regions. Nearly 20–30% of the produce is generally spoiled at post-harvest stages leading to losses. Michelin *et al.* (2012) described that agro industrial wastes are materials often loaded with cellulose and hemicelluloses and that use of such wastes for industrial interest is mainly due to their high availability at low/no cost. There is a need to preserve and utilized or transform the surplus and unutilized fruits to valuable products. Mango (*Mangiferaindica* L) is the most popular and the choicest fruit of India. A major portion (nearly 60–70%) of the total quantity produced is locally consumed and a sizable portion is exported to other countries (Reddy *et al.*, 2005; Reddy *et al.*, 2012).

Results of the present study indicated that peels of apples and mangoes, rind of watermelon and bagasse were best substrates for supporting growth of the bacterial isolates. While bagasse and rind of watermelon with addition of 0.1% watermelon pulp were best substrates for ethanol production at 5th day of incubation at 30°C. The result obviously indicated that the isolate AI-2 yielded higher amount of ethanol from the aqueous extract of watermelon, while the isolates AI-5 and AI-6 can be selected for the production of ethanol from bagasse plus watermelon aqueous extracts.

These bacterial isolates appeared highly valuable for their ability of conversion of the lignocellulosic materials into bioethanol. Production of bioethanol from low cost lignocellulosic materials which are most promising feed stock has been reported by various workers (Cardona *et al*., 2010; Balat, 2011; Sarkar *et al*., 2012).

Luz *et al.* (2008) descibed that all agri wastes have considerable sugar contents. Lignocellulosic materials contains large amounts of fermentable sugars such as xyose that cannot utilized by familiar brewing yeast. While the cellulolytic and ethanologenic bacteria isolated in this study indicated meaningful growth and ethanol production in bagasse and watermelon substrates. Gomathi *et al*. (2012) described that conversion of agri wastes into bioethanol will not only reduce global warming and green house effect but also decrease dependency on fossil fuels. Bagasse and watermelon are more abundant during summer season in Pakistan. Fish *et al.* (2009) documented that about 20% watermelon crop is left in the field because of surface damage. Abundant amount of such wastes are dumped around human residences, roads and near water bodies. Temperature is favorable for microbial growth in Pakistan so, these wastes cause loathsome dissemination and growth of pathogenic microorganisms.

One major problem of ethanol production from lignocellulosic materials is the availablilty of raw materials. As mentioned by Balat *et al.* (2008) and Gnansounou, (2010) that availability of raw material depends upon the geographical location. Luckily in Pakistan raw materials availability is always around the clock in every season. The substrates employed in this study that is peels of apples and Bagasse remain available in every season of the year but mango and watermelon are the crops of summer season. In Pakistan the summer season duration is much more thus the availability of raw materials, is not a problem. In one of the study Reddy *et al*. (2008) immobilized strain *Saccharomyces cerevisiae* on watermelon (*Citrullus vulgaris*) rind pieces and obtained ethanol yields from 9.5% to 12% v/v. Alcohol contents decreased after 36 hours of post fermentation at 30°C and 5pH due to utilization of sugar contents of the watermelon rind.

In this present study the bacterial isolate AI-6 gave ethanol yield up to 7.4% v/v in sugarcane bagasse and watermelon rind mixture following 144 hours of fermentation at 30°C and pH 7 in batch culture conditions. Carbohydrate and protein contents dropped down to 11.53±3.42µg/ml and 103.12±7.07µg/ml, respectively in batch fermentation. While 24.15±0.14 µg/ml and 172±14.86 µg/ml in fermenter after 144 and 96 hours of incubation respectively. Whereas these values further went down to 8.88±1.49µg/ml and 94.17±1.41 µg/ml in the batch fermentation while 16.40±0.51802 µg/ml and 106.35±15.88 µg/ml in fermenter at 168 hours of incubation. Thus for continuous yield the fresh medium is to be introduced to maintain the protein and carbohydrate contents around or above to 11.53±3.42 µg/ml and 103.12±7.07 µg/ml, respectively in batch fermentation and 24.15±0.14 µg/ml and 172±14.86 µg/ml in fermentater. The spent medium produce both in fed batch and continuous culturing would have to remove when yield approaches 7.4% v/v. In the present study bagasse and watermelon rind aqueous extracts yielded 7.4% ethanol after 144 hours of fermentation. Optimization of the isolate AI-6 revealed 7 pH, and 30°C for optimum temperature. The 30°C temperature appears less energy intensive in this country. Temperature is one of the most important factors affecting the ethanol production Reddy *et al*. (2008) described decrease in temperature below 30°C decreased yield of ethanol. While nutrophilic nature of the bacteria may permit their cultivation without any need of special pH adjustment. The present study for ethanol production from the agri food wastes was accomplished without any supplementation. Role of mineral/trace salt solutions and other growth promoting supplements in these media might be verified for enhanced bioethanol production.

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