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Original Research Article

Comparative studies on bioethanol production with immobilized cells of *Saccharomyces cerevisiae* local strain and *Saccharomyces cerevisiae* MTCC 170 by stationary and shaking fermentation methods

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A B S T R A C T

In the present study comparative analysis of bioethanol producing yeast strains was carried out. Two strains of yeasts were employed-*S. cerevisiae* MTCC 170 and locally isolated *S. cerevisiae* from tamarind fruit. Immobilization was carried out by Ca-alginate method and fermentations were performed by stationary and shaking methods. Immobilized beads were reused for four cycles of fermentation and process was monitored with respect to ethanol produced, left over sugar in the fermentation medium and fermentation kinetics were studied. The results were expressed as mean ± SD, the data was analyzed using one way ANOVA. The significant difference in ANOVA ($p<0.05$) was detected by the Fischer's Least significant Difference (LSD) multiple comparison test. Increase in ethanol production was observed up to third cycle of fermentation with highest ethanol production at 72 hr in third cycle of fermentation in both stationary and shaking fermentation methods followed by decrease in ethanol in fourth cycle of fermentation. Among the two strains used highest ethanol production of 75.66±0.57 g/l was recorded with immobilized yeasts of *S. cerevisiae* local strain than MTCC strain (59.66±0.57 g/l) in shaking method of fermentation. Results indicated that the growth and fermentation kinetics with the cells immobilized with alginate in shaking fermentation was faster than with stationary fermentation with the ethanol yield ($Y_{p/s}$= 0.48±0.02 g g^{-1}), glucose consumption (99.58±0.08 %), volumetric substrate uptake (Q_S= 2.04±0.03 g L^{-1} h^{-1}) and volumetric product productivity (Q_p,= 1.03±0.02 g L^{-1} $^{h-1}$). A statistically significant difference of $p < 0.05$ (Fischer's LSD test) was found on ethanol yield after 72 hr using immobilized yeast cells.

K e y w o r d s

Immobilization; stationary fermentation; shaking fermentation; *Saccharomyces cerevisiae;* calcium alginate entrapment.

Introduction

The increasing demand for ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives has necessitated increased production of this alcohol. Ethanol production is usually accomplished by chemical synthesis of petrochemical

substrates and microbial conversion of carbohydrates present in agricultural products. Owing to depleting reserves and competing industrial needs of petrochemical feedstocks, there is global emphasis in ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Brooks, 2008).

Bioethanol is the most widely used liquid biofuel. Its market is expected to reach $100x10^9$ liters in 2015 (Licht, 2006). The largest producers in the world are the United States, Brazil and China. In 2009, US produced $39.5x10^9$ liters of ethanol using corn as a feedstock while the second largest producer, Brazil created about $30x10^9$ liters of ethanol using sugarcane. China is a country that has invested much in the production of ethanol, and is nowadays one of the largest ethanol producers (Li, *et al*., 2009). Currently, almost all bioethanol is produced from grain or sugarcane (first generation bioethanol) (Cardona, 2010).

Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released CO_2 is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle (Wyman, 1999). Ethanol production process only uses only energy from renewable energy sources; no net CO_2 is added to the atmosphere, making ethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect (Foody B, 1988).

Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While attach cells in the natural habitat exhibit significant growth, the artificially immobilized cells are allowed restructure growth. Since the time, first reports of successful applications of immobilized cells in industrial applications, several groups over world have attempted whole cell immobilization as a viable alternative to conventional microbial fermentation (Prasad *et al*., 2009). Various immobilization processes has also triggered our interest in bioreactor design. Using immobilized cells, different bioreactor configuration were reported with variable success. The study on the physiology on immobilized cells and development on non-invasive measuring techniques have remarkably improve our understanding on microbial metabolism under immobilized state.

Many methods namely adsorption, covalent bonding, cross linking, entrapment and encapsulation are widely used for immobilization. These categories are commonly used in immobilized enzyme technology. However, due to completely different size and environmental parameters of the cells, the relative importance of these methods is considerably different. The criteria imposed for cell immobilization technique usually determine the nature of the application. Among the different cell immobilization techniques, entrapment in calcium alginate gel has been one of the most used matrices for whole cell entrapment due to its simplicity and non-toxic character. This simple and mild immobilization technique involves the drop-wise addition of cells suspended in sodium alginate onto a solution of calcium chloride whereon the cells are

immobilized in precipitated calcium alginate gel in the form of beads (Rosevear, 1984). Keeping in view the growing market of bioethanol fuel, advantages of ethanol as a clean burning fuel and necessity of a high ethanol yielding strain, we have carried out the present study with following objectives: a) to design a process which can reduce time and energy needed for inoculum development, b) to cut down the cost of recovery of the product, c) to reuse the cells for further fermentation cycles by the use of immobilization technique, d) to compare the stationary and shaking fermentation methods and e) to check the ethanol production efficiency of the locally isolated *S.cerevisiae* strain with a reference strain of *S.cerevisiae* MTCC 170.

Materials and Methods

Microorganisms and culture

Saccharomyces cerevisiae

The yeast *Saccharomyces cerevisiae* was isolated from tamarind fruit. Tamarind were obtained from a local fruit market in Hyderabad Metropolis, collected in sterile containers and transferred to laboratory. Tamarinds (~ 20g) were washed with sterile distilled water and washed water was plated onto YPD+C medium containing- Yeast extract (3.0g); Peptone (10.0g); Glucose (20.0g); Distilled water (1000ml); p^H (5.5) and Chloramphenicol (0.03%). The plates were incubated at 30^0C for 48 hrs. Colonies suggestive of yeasts were preliminary identified by microscopic examination for cell shape and budding formation. The species was identified as *Saccharomyces cerevisiae* by studying morphological, biochemical and physiological characteristics (Kregervan Rij, 1984).

Saccharomyces cerevisiae MTCC 170

This yeast culture was procured from MTCC (Microbial Type Culture Collection Centre and Gene Bank), Chandigarh, India. The yeast was cultured and maintained on YPD (Yeast extract, peptone and dextrose) agar media at 30^0C. In the present study *S.cerevisiae* MTCC 170 was used for comparative studies with locally isolated strain for ethanol production.

Inoculum Preparation

For inoculum preparation 100 ml YPD broth medium (as mentioned above without agar) was taken in two sterilized 250 ml Erlenmeyer flasks (sterilized at 121^0C for 20 min). The flasks were inoculated with loopful of *S.cerevisiae* and *S.cerevisiae* MTCC 170 cultures and incubated at 30^0C for 24 hrs at 120 rpm in an orbital shaking incubator (Kemi Pvt, Ltd, Kerala, India). Fifty (50 ml of each yeast inoculum (equivalent to 10% of fermentation medium) were separately immobilized with Ca-alginate as described in below section.

Cell Immobilization

To carry out immobilization, 2% of $CaCl_2$ solution was prepared and kept at 4^0C for chilling. 50ml each of previously grown cultures of *S. cerevisiae* and *S. cerevisiae* MTCC 170 in YPD broth were centrifuged at 5000 rpm for 15 minutes in refrigerated centrifuge (Kemi Pvt, Ltd, Kerala, India). The supernatant was discarded and the pellet was washed with saline water. Centrifugation was carried out again at 5000rpm for five minutes to obtain the final pellet that was washed, air dried and weighed. Two grams of sodium alginate was dissolved in hot water with constant

stirring on magnetic stirrer. After cooling sodium alginate solution, 2g of each yeast biomass was added to the slurry under stirring conditions for even dispersal. The slurry solution, with yeast biomass was dispersed drop wise with hypodermic syringe into 2% chilled $CaCl_2$ solution. Spherical beads (~3.0 mm diameter) were formed for both *S.cerevisiae* and *S.cerevisiae* MTCC170 which were washed with 0.5% chilled $CaCl_2$ solution at 4^0C for hardening. Finally these beads were washed with sterile distilled water to remove excess Ca^{2+} ions and un-entrapped cells, before being used for fermentation process.

Setting up of fermentation

For fermentative production of bioethanol 500ml yeast fermentation medium containing 15% glucose and p^H 5.5 was taken in two 1000ml Erlenmeyer flasks. The flasks were sterilized by steaming at 10 lbs for 20 minutes, cooled and inoculated with Ca-alginate beads. Fermentation was carried out by stationary and shaking method at 30^0C. For the shaking method an orbital shaking incubator set at 100 rpm and 30^0C temperature was employed. Both stationary and shaking fermentation were carried out for 72 hrs. After 72 hrs of fermentation the immobilized beads were separated from exhausted medium by filtration method and replaced in fresh fermentation medium under aseptic conditions and incubated for another 72 hrs for fermentative production of bioethanol. In this way four (4) cycles of fermentation were carried out with immobilized beads.

Cell leakage

The cells leaked from the gel matrix into the fermentation medium were determined by plate counting using yeast extract-glucose – salt – agar (YGSA medium) incubated at 30^0C for 24h.

Analytical methods

In each fermentation cycle, at 24 hr interval fermented broths (in triplicates) were removed and analyzed for left over sugar and ethanol content. The estimation of left over sugar was based on the dinitrosalicylic acid (DNS) method (Miller, 1959). A double beam UV/VIS-scanning spectrophotometer was used for measuring absorbance at 575 nm. Ethanol was determined with good precision by oxidation with acid dichromate solution (Caputi *et al*., 1968) and absorbance was measured at 660 nm. The biomass of calcium alginate immobilized beads was determined by dissolving the gel beads in a 4% (w/v) EDTA solution, the beads were aseptically crushed by a sterile glass rod with sterile water. Finally the reading was taken at 550 nm against a suitable curve of absorbance versus dry weight (Behera *et al*., 2010). The fermentation kinetics was studied as per the formulae given by Bailey and Ollis (1986).

Statistical analysis

All experiments were carried out in triplicates (n=3) and values were represented as mean ± SD. The data of ethanol yield using immobilized cells were analyzed using one way ANOVA. The significant difference in ANOVA ($p<0.05$) was detected by the Fischer's Least significant Difference (LSD) multiple comparison test which was applied to compare the factor level difference. The analysis was performed using SPSS software.

Results and Discussion

In the present work comparative studies were performed on bioethanol fuel production by stationary and shaking fermentation methods using immobilized bead of *S.cerevisiae* local strain and *S.cerevisiae* MTCC 170 and following results were obtained.

Stationary fermentation

The results obtained for stationary fermentation with immobilized yeasts are shown in Table-1. Increase in bioethanol production was observed for reference beads of *S.cerevisiae* MTCC 170 with increase in fermentation time with highest yield (45.66±0.57g/l) at 72 hrs of fermentation. When beads were re-used for second fermentation cycle, ethanol production increased than first cycle with a yield of (51.66±0.57g/l). This pattern of increase in bioethanol production continued upto third cycle of fermentation after which decrease in ethanol production was observed. Among all cycles of fermentation highest yield was recorded in third fermentation cycle at 72 hrs with a production of 53.66±0.57 g/l. When the results are compared with local strain of *S.cerevisiae* higher ethanol production was observed than reference strain of MTCC 170 at 72 hrs of fermentation, with a yield of 60.2±0.20 g/l in first cycle of fermentation. The pattern of bioethanol production was similar to MTCC 170 strain where in highest production (70.33±0.57) was recorded in third cycle of fermentation at 72 hrs. After third cycle decrease in bioethanol production was observed (Table-1).

Along with increase in bioethanol production there was simultaneous decrease in left over sugar in the fermentation medium as shown in Table-1. A similar pattern of decrease in left over sugar was observed for both yeast strains with higher decrease in left over sugar recorded for *S.cerevisiae* local strain. This corresponds to higher ethanol production by local strain which is higher than MTCC 170 strain. The data obtained for left over sugar is used for estimating sugar utilized, theoretical yield and fermentation kinetics.

Shaking fermentation

In the present study higher levels of ethanol production were observed in shaking fermentation than stationary fermentation method for both yeast strains. Immobilized beads performed better with each cycle of fermentation upto third cycle after which decline in production of bioethanol was observed. Compare to MTCC 170 strain higher ethanol production was recorded for *S.cerevisiae* local strain which yielded 75.66±0.57 g/l of bioethanol in third cycle of fermentation at 72 hrs which is much higher than 59.66±0.57 g/l produced by MTCC strain under same fermentation conditions for same time. The results for shaking fermentation are shown in Table-2.

The results for left over sugar in shaking fermentation are depicted in Table-2. In each fermentation cycle constant decrease in left over sugar was observed in the fermentation medium. This pattern of left over sugar was recorded in all four cycles of fermentation with immobilized beads for both yeast strains. In shaking fermentation also decrease in left over sugar concentration is well complemented with simultaneous increase in bioethanol production except for fourth cycle in which decrease in ethanol production was recorded for both the yeast strains.

The overall performance of reference strain *S.cerevisiae* MTCC 170 with respect to ethanol produced and left over sugar in stationary and shaking fermentation methods is depicted in Figure-1. The figure highlights the improved performance of immobilized beads in shaking fermentation with highest peak of ethanol fermentation in third cycle and also sequential decrease in left over sugar in fermentation media in all four cycles of fermentation.

The higher bioethanol production ability of locally isolated strain of *S. cerevisiae* in stationary and shaking fermentation methods is shown in Figure-2. The peak of ethanol produced at 72 hrs of fermentation in third cycle is clearly highlighted in both stationary and shaking methods. In the present study growth and fermentation kinetics were studied for both stationary and shaking fermentation. The final ethanol concentration (*P*) recorded with immobilized beads in shaking fermentation with *S. cerevisiae* local strain was much higher (75.66±0.57 g/l) than that of MTCC 170 strain (59.5±0.50 g/l). The other parameters are shown in Table-3.

In the present study repeated batch fermentation for bioethanol production was carried out with immobilized cell beads of *S. cerevisiae* locally isolated strain and *S. cerevisiae* MTCC 170 in order to examine the advantages of immobilization and also to test the ethanol production ability of locally isolated strain. The parameters used during this study were: two methods of fermentation-stationary and shaking methods, incubation period with two strains of immobilized yeast cells, reuse of immobilized cells, initial P^H, incubation temperature, initial sugar concentration and volume of fermentation medium.

The parameters studied for calculating fermentation kinetics were final ethanol concentration (P, g L^{-1}), final biomass concentration (X, g L^{-1}), cell yield ($Y_{x/s}$, g/g), ethanol yield ($Y_{p/s}$ g g^{-1}), volumetric substrate uptake (Q_S, g L^{-1} h^{-1}), volumetric product productivity (Q_p, g L^{-1} h^{-1}) and glucose consumption (%).

In the immobilization process productivity depends upon various factors like size of inoculum, type of microorganisms, nature of the substrate and the type of the carrier material used for immobilization. A number of carrier materials- agar-agar, Ca-alginate, k-carrageenan etc. have been used for entrapping microbial cells for production of various metabolites like ethanol (Chandel *et al*., 2007), organic acids (John *et al*., 2007), aminoacids-glutamic acid (Pasha *et al*., 2011) and enzymes (Dhanasekaran *et al*., 2006). Among these, entrapment in Ca-alginate beads is found to be most suitable in majority of studies as the matrix is cost effective, procedure is simple and easy to handle and the alginate carrier results in enhanced activities of yeasts compared to other carriers (Najafpour *et al*., 2004 and Ciesarova *et al*., 1998), hence alginate immobilization was used in the present study for the production of ethanol by repeated batch fermentation. Reports have been published on the production of ethanol under stationary conditions (Roukas, 1996). In the present study both stationary and shaking methods were used

Table.1 Ethanol produced and Left over Sugar (LOS) in Stationary Fermentation Method (g/l)

Fermentation Cycles	Time in hrs	Ethanol produced in Stationary fermentation (g/l)		LOS in fermentation media (g/l)	
		S. cerevisiae MTCC 170	*S. cerevisiae* local strain	*S. cerevisiae* MTCC 170	*S. cerevisiae* local strain
Cycle-I	**24 hrs**	35.33±0.28	35.66±0.57	49.66±0.57	51.66±0.57
	48 hrs	40.33±0.57	51.33±0.28	39.66±0.57	26.16±0.28
	72 hrs	45.66±0.57	60.20±0.20	30.16±0.28	18.16±0.28
Cycle-II	**24 hrs**	40.53±0.41	50.33±0.57	39.83±0.28	27.66±0.57
	48 hrs	48.66±0.28	58.36±0.15	27.66±0.57	20.16±0.28
	72 hrs	51.66±0.57	64.66±0.57	23.50±0.50	16.43±0.40
Cycle-III	**24 hrs**	40.66±0.57	51.66±0.57	38.63±0.32	23.50±0.50
	48 hrs	49.63±0.32	60.66±0.28	27.16±0.28	17.66±0.57
	72 hrs	53.66±0.57	70.33±0.57	21.66±0.57	11.66±0.57
Cycle-IV	**24 hrs**	38.33±0.57	50.66±0.57	48.16±0.28	25.50±0.50
	48 hrs	40.33±0.57	54.66±0.57	34.66±0.57	20.66±0.57
	72 hrs	38.66±0.57	57.66±0.57	29.66±0.57	18.66±0.57

Values are represented as mean ± SD

Table.2 Ethanol produced and Left over (LOS) Sugar in Shaking Fermentation Method (g/l)

Fermentation Cycles	Time in hrs	Ethanol produced in Shaking fermentation (g/l)		LOS in fermentation media (g/l)	
		S. cerevisiae MTCC 170	*S. cerevisiae* local strain	*S. cerevisiae* MTCC 170	*S. cerevisiae* local strain
Cycle-I	**24 hrs**	37.66±0.57	40.33±0.57	49.66±0.57	45.83±0.28
	48 hrs	44.66±0.28	52.40±0.13	31.66±0.57	23.66±0.57
	72 hrs	48.33±0.28	61.66±0.57	27.66±0.57	15.83±0.28
Cycle-II	**24 hrs**	41.66±0.57	46.55±0.57	38.16±0.28	31.50±0.50
	48 hrs	48.70±0.26	54.40±0.20	28.40±0.40	22.16±0.28
	72 hrs	53.66±0.57	64.66±0.22	21.83±0.76	17.16±0.28
Cycle-III	**24 hrs**	44.43±0.20	45.16±0.28	31.66±0.57	31.66±0.57
	48 hrs	51.66±0.57	64.66±0.57	23.83±0.28	20.16±0.28
	72 hrs	59.66±0.57	75.66±0.57	19.16±0.28	0.45±0.05
Cycle-IV	**24 hrs**	41.66±0.28	43.33±0.28	39.16±0.28	31.50±0.50
	48 hrs	45.66±0.28	51.33±0.57	30.16±0.28	24.83±0.28
	72 hrs	47.83±0.76	60.66±0.28	27.16±0.28	18.33±0.28

Values are represented as mean ± SD

Figure.1 Overall performance of *S.cerevisiae* MTCC 170 in stationary and shaking fermentation methods

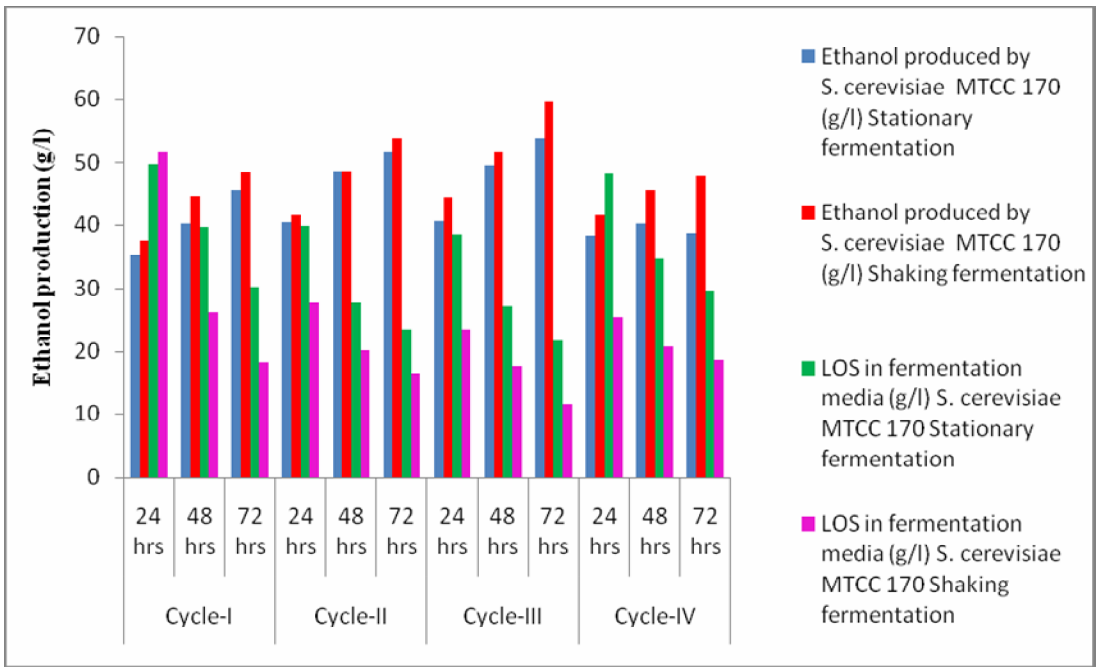

Figure.2 Overall performance of *S.cerevisiae* locally isolated strain in stationary and shaking fermentation methods

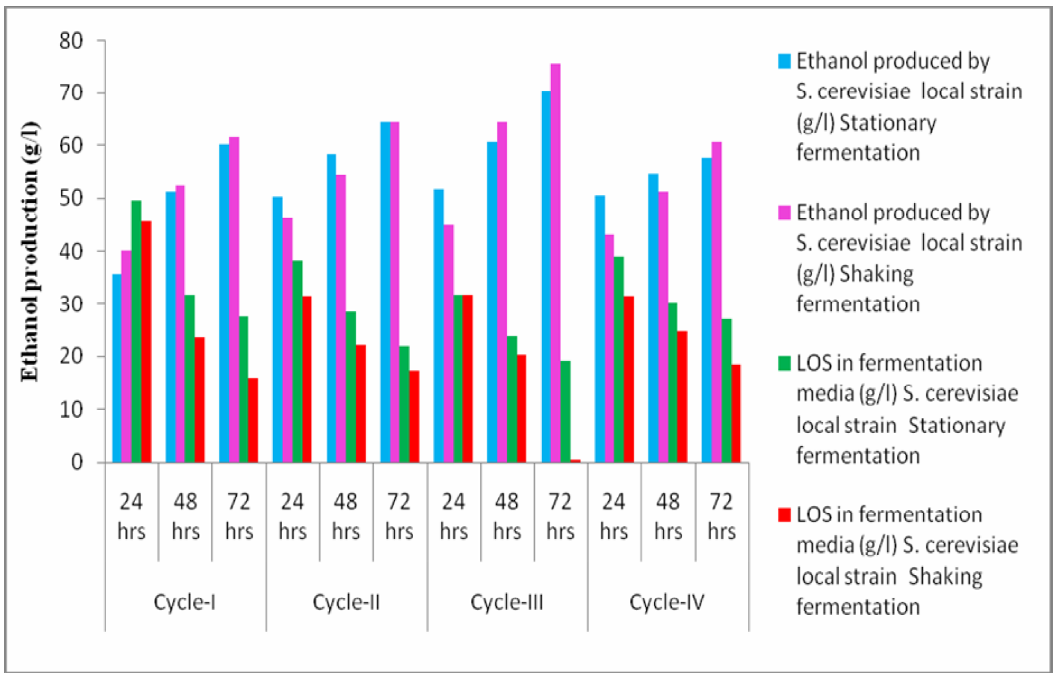

Table.3 Growth and Fermentation kinetics of Ca-alginate immobilized cells of *S. cerevisiae* MTCC 170 and *S. cerevisiae* local strain

Paramaters	Stationary Fermentation		Shaking Fermentation	
	***S. cerevisiae* MTCC 170**	***S. cerevisiae* local strain**	***S. cerevisiae* MTCC 170**	***S. cerevisiae* local strain**
Final ethanol (P, g L^{-1})	53.66±0.28	69.5±0.50	59.5±0.50	75.66±0.57
Final Biomass concentration (X, g L^{-1})	2.31±0.01	2.94±0.02	2.48±0.03	3.17±0.02
Cell yield ($Y_{x/s}$, g/g)	1.31±0.01	1.53±0.01	1.35±0.01	1.54±0.005
Ethanol Yield ($Y_{p/s}$ g g^{-1})	0.41±0.01	0.46±0.02	0.42±0.02	0.48±0.02
Volumetric substrate uptake (Q_S, g L^{-1} h^{-1})	1.75±0.01	1.91±0.01	1.82±0.02	2.04±0.03
Volumetric product productivity (Q_p, g L^{-1} h^{-1})	0.73±0.02	0.96±0.01	0.82±0.02	1.03±0.02
Glucose consumption (%)	85.44±0.09	91.5±0.50	87.34±0.15	99.58±0.08

for comparative analysis of bioethanol produced by immobilized yeasts beads.

Cultural conditions for ethanol production vary from strain to strain and also depend on the type of process adopted. Among all the yeasts, *S. cerevisiae* was proved more successful for ethanol production as compared to other species (Ergun and Ferda, 2000). This is due to the fact that some species adopt different metabolic pathways by having special genes or special enzymes such as invertase genes and invertase enzymes respectively for the conversion of sugars to ethanol or other metabolites (Fregonesi *et al*., 2007).

Results presented in Table-3 indicated that the growth and fermentation kinetics with the cells immobilized with alginate in shaking fermentation was faster than with stationary fermentation and in this system higher ethanol concentrations were achieved. The final ethanol concentration (P) obtained in shaking fermentation (75.66±0.57g/l) for *S. cerevisiae* local strain was much higher than stationary fermentation of MTCC strain (53.66±0.28 g/l). This higher concentration of ethanol was due to higher percentage of sugar consumed in shaking fermentation (99.58±0.08%) than stationary fermentation (85.44±0.09%) by yeast cells. Similarly, enhancement in final biomass concentration (X=3.17±0.02gL^{-1}) and cell yield ($Y_{x/s}$=1.54±0.005g/g) for *S. cerevisiae* local strain was recorded in shaking fermentation than stationary fermentation (X=2.31±0.01gL^{-1} and $Y_{x/s}$=1.31±0.01g/g) of MTCC strain. The volumetric product productivity (Q_p=1.03±0.02g L^{-1} h^{-1}) and ethanol yield ($Y_{p/s}$ =0.48±0.02 g g^{-1}) obtained with immobilized cells of *S. cerevisiae* local strain in shaking fermentation was found to be 1.41 and 1.14% respectively higher than that of Q_p (0.73±0.02 g L^{-1} h^{-1}) and $Y_{p/s}$ (0.41±0.01 g g^{-1}) of immobilized cells of MTCC strain in stationary fermentation.

In our earlier studies, higher ethanol concentration was recorded in stationary fermentation than shaking fermentation when the free cells of *S. cerevisiae* were

used in place of immobilized beads (Ali *et al*., 2011a & b). Whereas in the present study shaking fermentation resulted in more ethanol production than stationary fermentation, this is mainly due to cell immobilization which offers enhanced substrate utilization and fermentation productivity, feasibility of continuous processing by reuse of immobilized beads which reduces the time and media required for inoculum preparation. Immobilization of cells offers other numerous advantages like lower cost of recovery, recycling and downstream processing. It also eliminates washout problems at high dilution rates.

The results show that there was 85.0 to 99.0 % sugar utilization at the end of 72 hr incubation period using immobilized yeast cells in both methods of fermentation. Further, there was a statistically significant difference of $p< 0.05$ (Fischer's LSD test) found on ethanol yield after 72 hr using immobilized yeast cells. In general, 10-20% increase in ethanol yield has been reported in immobilized cells entrapped in matrices such as Ca-alginate, luffa sponge, agar agar etc., over free cells for various bio-products production (Amutha, 2001; Kourkoutas, 2004). In the present study, the immobilized cells were further reused for three more times limiting the duration of each fermentation cycle up to 72 hr as most of the sugar in fermentation media was consumed during this period. The cells not only survived but also were active physiologically yielding ethanol 61.66±0.57, 64.66±0.22 and 75.66±0.57 g/l respectively in first, second and third cycle of 72 hr shaking fermentation. After third fermentation cycle ethanol yield decreased in fourth cycle of fermentation, this decrease in ethanol concentration is due to leakage of cells from the Ca-alginate matrix (0.05-0.15 mg/ml). The leakage of cells from immobilized support was negligible (< 5%), hence the observed ethanol production was due to action of immobilized cells.

Based on the results obtained we can conclude that immobilized cells produces higher levels of bioethanol than free cells with better fermentation kinetics and Ca-alginate is one of the best carrier for immobilization having the ability to support stable fermentation for immobilized beads up to three cycles of fermentation. We can also conclude that locally isolated *S. cerevisiae* strain used in the present study is a high bioethanol producing strain than the reference strain of *S. cerevisiae* MTCC 170 and shaking fermentation is the ideal method for production of bioethanol when immobilized yeasts are employed.

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