Production, Purification and Characterization of of β-galactosidase Synthesized by Bacillus safensis (JUCHE 1)

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Abstract- In present investigation production, purification and characterization of β-galactosidase synthesized by Bacillus safensis (JUCHE 1) has been considered as a great challenge. Optimum concentration of lactose, nitrogen sources, namely tryptone in modified de-Mann Rogosa Sharpe (MMRS) medium were found 20 g.L⁻¹ and 10 g.L⁻¹ respectively. Optimum temperature, pH and stirrer speed for microbial growth were found 37°C, 7.0 and 150 r.p.m. respectively. To isolate intracellular β-galactosidase sonication was adopted and optimum sonication time was found 300 seconds with inter stage cooling for 10 seconds after an operating time of 30 seconds. 28 hours harvested broth was centrifuged and cell pellet was used to isolation of intracellular β-galactosidase. Intracellular fluid was subjected into the dead end membrane module fitted with 0.2*10⁻⁶m microfiltration (MF) membrane and 300 kDa Polyether sulphone (PES) made ultrafiltration (UF) membrane in a series way. Optimum Stirrer speed, trans membrane pressure (TMP), volume concentration factor (VCF) and membrane rotation were found 150 r.p.m., 294.2 kPa, 3 and 80 r.p.m. respectively. Finally, 45% (w/v) addition of ammonium sulphate β-galactosidase was purified. β-galactosidase was characterized considering ortho-Nitrophenyl-β-galactoside (ONPG) and lactose as a substrate.

Key words: Bacillus safensis, Growth kintics, β-galactosidase production, β-galactosidase purification, β-galactosidase characteristcs

I. INTRODUCTION

Biopharmaceuticals, a new category of drug originated from biological sources, have been emerged by the immense development in the field of biotechnology. The therapeutics potential biomolecules forms the basis of extensive development of the microbial synthesized enzymes and metabolites [1].

The lactose hydrolyzing enzyme, β-galactosidase (β-galactosidase galacto hydrolase, trivially lactase) has long been accepted as an important ingredient in food processing industries. β-galactosidase catalyzes hydrolysis of lactose to produce glucose and galactose and in some cases it takes part in transgalactosylation reaction, that produces GOS (for example, β1→4 Gal (β1→6) [2]. In dairy industry, β-galactosidase could be used to prevent crystallization of lactose, to improve sweetness, to increase the solubility of milk product, to prepare low lactose containing food products for low lactose tolerant people and for the utilization of cheese whey, which would otherwise be an environmental pollutant [3]-[5]. In most of the human races β-galactosidase are lost during the first or second span of life. The hypolactasia (lactose intolerance) more precisely, lactose mal-digestion is caused due to insufficient synthesis of lactose cleaving enzyme β-galactosidase, in the brush border membrane of the mucosa of the small intestine. Only people of Northern European origin, their overseas descendents and some isolated, African and Indian communities maintained a high intestinal lactase activity throughout their life. According to reported literature data, the global prevalence of lactose mal-digestion is above 50% in South America, Africa, and almost 100% in some Asian countries like China. In the United States, the prevalence is 15% among whites, 53% among Mexican-Americans and 80% in the Black population. In Europe it varies from around 2% in Scandinavia to about 70% in Sicily. Australia and New Zealand have prevalence of 6% and 9% respectively [6]-[8]. Therefore, edible grade lactase synthesis using bacterial consortium ensures the proper consumption of lactose derivatives, which otherwise may lead to lactose intolerance.

Presences of the lac operon in Bacillus sp. including lactic acid bacteria, has been potential for synthesizing of intracellular β-galactosidase (E.C.3.2.1.23) trivially known as lactase. It catalyses disaccharide milk sugar lactose into monosaccharide glucose and galactose, and in some cases lactase is able to catalyze transglycosylation reactions. Regarding the investigation on structure of lac operon of Escherichia coli, its regulation and biosynthesis of lactase by different microorganisms were performed for more than last two decades [9]–[14]. Different separation techniques already attempted for purification of β-galactosidase from crude extract. β-galactosidase was purified 36.2-fold by ZnCl₂ precipitation, ion exchange, hydrophobic interaction and gel
filtration chromatography with an overall recovery of 12.7% [15]. β-galactosidase was isolated from two different species of Lactobacillus reuteri L103 and L461 and subsequently, β-galactosidase was purified by ammonium sulfate fractionation, hydrophobic interaction, and affinity chromatography. The two enzymes were purified approximately 16- and 50-fold from the crude cell extracts with an overall yield of 7 and 12% [16]. Three isoforms of β-galactosidase, 212 kDa (I), 145 kDa (II) and 86 kDa (III) were separated by gel permeation chromatography, affinity chromatography and ammonium sulphate precipitation and subsequently their characterization was carried out considering o-nitrophenyl-β-D-galactopyranoside (ONPG) and lactose as a substrate [17]. Therefore, β-galactosidase production through fermentative process using bacterial sp. and subsequently its purification will be useful both in dairy industries, food industries as well as in pharmaceutical industries and a large economic boom could be expected.

The present work has been focused on synthesis of intracellular β-galactosidase using isolated Bacillus safensis (JUCHE 1) in a lab-scale fermentor by batch mode and subsequently it’s purification by UF and salting out technique. Bacillus safensis (JUCHE 1) is a unique microorganism, isolated in our laboratory and is capable of producing thermostable β-galactosidase amylase, cellulase, protease. Two types of microbial growth medium, namely lactose broth and MMRS medium were tested for microbial growth and β-galactosidase production. Effects of concentrations of lactose in suitable growth medium, proteins such as peptone, tryptone and casein, bulk concentration and different operating conditions such as stirrer speed, pH and temperature were investigated individually for optimizing microbial growth, as well as β-galactosidase production. Microbial growth kinetic parameters, namely, maximum specific growth rate (μmax), Monod constant (Ks), yield coefficient (Yx/s) were also evaluated. Twenty eight hours harvested culture was considered for purification of intracellular β-galactosidase. Intracellular fluid of microbial biomass was obtained by sonication and sonication time was optimized. Size exclusion based UF followed by salting out by ammonium sulphate were adopted for purification of β-galactosidase. In the present investigation, a dead end UF membrane module has been considered for β-galactosidase purification. In UF technique different operating parameters, such as, TMP (98.07 kPa to 392.27 kPa), stirrer speed (50 r.p.m. to 200 r.p.m.), membrane rotation speed (40 r.p.m. to 100 r.p.m.) and VCF (2 to 4) were varied. Under the present investigation, four stage diafiltration (DF) have been carried out for purification of β-galactosidase. Hydrodynamic studies of dead end membrane module under different operating condition were also investigated. Finally, β-galactosidase was purified by ammonium sulphate precipitation followed by dialysis. β-galactosidase was characterized with respect to maximum reaction velocity (vmax), Michaelis Menten constant (Km), optimum reaction temperature and pH considering ortho-Nitrophenyl-β-galactosidase (ONPG) and lactose as a substrate.

II. MATERIAL AND METHODS
2.1. Materials

Lactose, glucose, tryptone, yeast extract and beef extract, procured from HIMEDIA, Mumbai, India; di-potassium hydrogen phosphate, sodium acetate, manganese sulfate, magnesium sulfate, Folin-Ciocalteau reagent and acetonitrile procured from Merck, Mumbai, India; ammonium citrate, sodium potassium tartrate, sodium carbonate, copper sulfate, hydrogen chloride and sodium hydroxide procured from Ranbaxy, Mumbai, India; ONPG, phosphate buffer, tris buffer, citrate buffer procured from Sigma Aldrich, USA were used.

2.2. Equipment

A B.O.D incubator cum shaker (Sartorius AG, Göttingen, Germany), an indigenous microfiltration unit along with cellulose acetate membrane of 47 mm diameter and 0.45 μm pore size (Sarada Chemicals, Kolkata, India), a UV laminar flow hood, a hot air oven (Bhattacharya & Co., Kolkata, India), a magnetic stirrer, a cold centrifuge (C-24) (Remi Instruments Ltd., Mumbai, India), an autoclave (G.B. Enterprise, Kolkata, India), a sonicator (Sartorius AG, Göttingen, Germany) and a 5 L Jar fermenter (Eyla, Japan) were used.

2.3. Analytical instruments

A digital pH meter, a digital weighing machine (Sartorius AG, Göttingen, Germany), a high performance liquid chromatography (HPLC) (Perkin Elmer, Series 200) and a double beam UV-VIS spectrophotometer (SPECTRASCAN UV 3600-Chemitro) were used. The HPLC system was associated with RI detector and Spheri 5 amino column (5 μm, 4.6 mm X 220 mm). The temperature of the HPLC was maintained at 298 K. Acetonitrile 75% (v/v) was used as the mobile phase at a flow rate of 1.67*10^-5 m^3/s for carbohydrate analysis [18].

2.4. Microorganism

An isolated strain of facultative anaerobic microbial strain, namely, Bacillus safensis (JUCHE 1), (Patent: 755/KOL/2011), was used in the present investigation. Although a facultative anaerobe, growth of Bacillus safensis (JUCHE 1), was favored under aerobic condition. Therefore, aerobic condition was maintained during all experiments described in the following section.

2.5. Methodology

2.5.1. Microbial growth medium preparation

MMRS medium and lactose broth were used separately for microbial growth medium as well as β-galactosidase synthesis. The MMRS medium contained per litre: tryptone 10.0 g, beef extract 10.0 g, yeast extract 5.0 g, ammonium citrate 2.0 g, sodium acetate 5.0 g, magnesium sulfate 0.1 g, manganese sulfate 0.5 g, dipotassium phosphate 2.0 g and lactose 20.0 g. The lactose broth contained per litre: lactose 10.0 g, yeast extract 5 g and sodium chloride 5 g. pH of the both medium were adjusted at 7.0 by 0.1 N sodium hydroxide and 0.1 N hydrogen chloride. Sterilization of all components
of the growth medium except lactose was done in an autoclave at 121°C for 15 minutes. Lactose was sterilized using indigenous microfiltration unit equipped with cellulose acetate membrane (47 mm diameter, pore size-0.45 µm) for its sensitivity towards high temperature.

2.5.2. Batch Studies

To determine the time history of microbial growth as well as synthesis characteristics of β-galactosidase several batch experiments were conducted by 5 L jar fermenter (working volume 2 L). Batch experiment were performed individually by lactose broth and MMRS medium. Effects of concentrations of lactose in microbial growth medium, influences of protein, namely, peptone, tryptone and casein in microbial growth medium, different operating conditions such as stirrer speed, pH and temperature. Samples were withdrawn from the bioreactor through a capillary needle into evacuated sample tubes. The sample tubes were immediately placed in a refrigerator at 4°C for inactivation of cellular enzymes. The sample tubes were immediately centrifuged by REMI made centrifuge at 10,000 rpm, 4°C for 15 minutes individually and the separated bacterial biomass were washed with phosphate buffer (pH 7). Subsequently, sonication was performed for 24 hours by 1.0 kD dialysis membrane with reference of 0.05 M sodium phosphate buffer (pH 7).

Finally β-galactosidase was purified by ammonium sulphate precipitation followed by dialysis. Ammonium sulphate was added gradually into partially purified β-galactosidase at constant stirring condition. Target enzyme pellet was dialyzed for 24 hours by 1.0 kD dialysis membrane with reference of 0.05 M sodium phosphate buffer (pH 7).

2.5.3. β-galactosidase purification

In present investigation, 28 hours old culture broth was considered for enzyme purification. Harvested culture broth was centrifuged at 12,000 r.p.m. for 15 minutes at 4°C. The pellet of microbial biomass was washed twice with distilled water and finally pellet was resuspended in 0.05 M sodium phosphate buffer at pH 7.0. Subsequently, sonication was adopted for obtaining intracellular fluid from cell biomass. Cell pellet was sonicated at 16 kHz with a probe of 9.5 mm using a constant power source of 240 V. Different sonication time, such as, 60-300 second were varied for optimization of the process. Inter stage cooling for 10 seconds was maintained after an operating time of 30 seconds. Optimum sonication time was considered with respect to highest enzymatic activity. Cell suspension was removed by centrifugation (10,000 r.p.m. for 20 minutes) and supernatant was collected. Intracellular fluid was subjected into dead end centrifugation (10,000 r.p.m. for 20 minutes) and VCF (2 to 4) were varied. In present investigation, four stage DF have been carried out for purification of β-galactosidase. Permeate volumes of dead end membrane module was collected at constant time intervals and permeate flux was calculated according to the following correlation.

\[ \text{Flux} = \frac{\text{Effective membrane surface area} \times \text{Time difference}}{\text{Volume fraction}} \]

2.5.4. Analytical methods

2.5.4.1. Determination of microbial bio mass concentration

Concentrations of microbial bio mass in broth of individual batch type were determined by dry weight method. In this method 20 ml broth, enriched with bacterial bio mass were centrifuged by REMI made centrifuge at 10,000 rpm, 4°C for 15 minutes individually and the separated bacterial bio mass were washed with phosphate buffer (pH 7.0) solution. The washed bio mass was transferred into a pre-weighed aluminum cup individually and was dried at 80°C for 0.5
hours. The exact weight of bacterial biomass is determined by subtracting the weight of dry cup from that of the cup containing dry bacterial mass. Cell growth was determined by measuring the absorbance at 660 nm using a spectrophotometer also.

2.5.4.2. Carbohydrate Estimation
HPLC was used to determine the concentrations carbohydrates.

2.5.4.3. Enzymatic activities of intracellular fluid
Sonicated bacterial mass was centrifuged and supernatant was assayed for β-galactosidase according to Miller’s method [19]. ONPG was used as substrates for β-galactosidase. Intracellular protein concentration was estimated by Lowry assay method considering bovine serum albumin as a standard protein [20]. Specific activities of intracellular fluid with respect to β-galactosidase were determined [21]. In this investigation, β-galactosidase was characterized with respect to maximum reaction velocity ($v_{\text{max}}$), Michaelis Menten constant ($K_m$), optimum reaction temperature and optimum reaction pH considering ONPG and lactose as a substrate. ONPG and lactose concentration were varied in the range of 5 mM to 40 mM and 11 mM to 30 mM respectively. Initial reaction velocities corresponding to different initial lactose or ONPG concentrations were used to determine the kinetic parameters namely, maximum reaction velocity ($v_{\text{max}}$), Michaelis Menten constant ($K_m$). Effects of temperature on enzymatic activity were evaluated at different temperature ranging from 20°C to 60°C. Effects of pH on enzymatic activity were evaluated at different pH of substrate ranging from 4 to 8.5.

III. RESULTS AND DISCUSSION
In the present investigation economic production and purification of β-galactosidase have been considered as a great challenge. Batch experiments were performed by facultative anaerobic microbial consortium Bacillus safensis (JUCHE 1) using lab scale 5 lit jar fermentor. Composition and pH of microbial growth medium, incubation temperature, stirrer speed were varied to assess the microbial growth and β-galactosidase production.

In Fig. 1, biomass concentration is plotted against incubation time considering composition of microbial growth medium as a parameter. It is observed that microbial growth is high in MMRS medium compare with lactose broth. It may be justify by the fact that presence of different metallic and non metallic ions in MMRS medium have positive influence on microbial growth. Therefore, MMRS medium was used as microbial growth medium for further experiment.

In Fig. 2 biomass concentration is plotted against incubation time considering concentration of lactose in microbial growth medium as a parameter. Concentration of lactose in microbial growth medium was varied in the range of 4 g.L$^{-1}$ to 40 g.L$^{-1}$.

The analysis of the figure, it is evident that growth of microbial consortium is strongly influenced by the lactose concentration up to 20 g.L$^{-1}$, after which no further increase of growth is observed with the increase of lactose concentration. This may be due to the presence of steric hindrance of lactose specific enzyme, caused by overloading of lactose molecules at higher rate of lactose concentration. Microbial growth kinetic parameters namely, Maximum specific growth rate ($\mu_{\text{max}}$), Monod half saturation constant ($K_s$), yield coefficient ($Y_{X/S}$) and maintenance coefficient ($m_s$) are evaluated and
those are 0.75 h⁻¹, 12.0 g.L⁻¹, 0.1 and 0.688 h⁻¹ respectively. In Fig. 3, biomass concentration is plotted against incubation time to study the influence of nitrogen sources on microbial growth. It is observed that tryptone, commonly known as casein hydrolysate has the best influence on microbial growth compare with other two nitrogen sources such as peptone and casein. It may be justified by the fact that nature of native nitrogen sources has positive influence on microbial growth.

In Fig. 5, biomass growth is plotted against incubation time considering stirrer speed as a parameter. It is observed that at 150 rpm microbial growth is high compare to below stirrer speed. It may be justified by the fact that microbial growth has affinity towards aerobic condition.

Initial pH of the microbial growth medium was varied in the range of 4.5-12. In Fig. 4, biomass growth is plotted against incubation time considering pH of the medium as a parameter. Optimum pH for microbial growth is found 7 when other operating parameters were remain unchanged.

In Fig. 6, microbial growth is plotted against incubation time considering incubation temperature as a parameter and optimum temperature of microbial growth is found 37°C.

The pattern of time histories of residual lactose concentration is shown in Fig. 7, are in agreement with trend of biomass concentration shown in Fig. 2. It is observed that concentration of lactose in MMRS medium are reduced monotonically.
In Fig. 8, activities of intracellular β-galactosidase μmol.min⁻¹.mg protein⁻¹ is plotted against sonication time and optimum sonication time is found 300 seconds with inter stage cooling for 10 seconds after an operating time of 30 seconds.

In Fig. 9, β-galactosidase activity (μmol/mg/min) has been plotted as a function of incubation time using initial lactose concentration (4 g.L⁻¹ – 40 g.L⁻¹) as a parameter. It is observed that specific activities of intracellular β-galactosidase is increased with incubation time and after 28 hour it is declined.

A dead end membrane module equipped with magnetic stirrer, membrane rotating device and pressurized with nitrogen gas cylinder has been used for purification of β-galactosidase from crude extract. Bacterial intracellular fluid is a mixture of different enzymes, metabolites and their derivatives. Therefore, purification of a particular enzyme fraction requires a very fine-tuned high precision technique. Different processes, such as size exclusion based UF techniques, salting out and dialysis were selected for this operation to provide a satisfactory β-galactosidase yield. Purification characteristics and β-galactosidase activity were investigated under different process parameters.

Bacterial intracellular fluid was fed into the membrane module for partial purification. After membrane compaction, series of water runs were taken to evaluate membrane hydraulic resistance Rm that is found to be \( (5.05 \times 10^8 \pm 1.5 \times 10^{10}) \) m⁻¹ for 300 kDa membrane. Centrifugation (10,000 r.p.m., 4°C, 15 minutes) followed by MF were employed for separation cell suspension from the supernatant. Permeate of MF membrane was used as a feed of UF technique. In Fig. 10, permeate flux at the steady state has been shown for 300 kDa membrane at different VCF keeping all other parameters constant. Under present investigation, VCF was maintained 2 to 4 by de-ionized water. It is observed that permeate flux is increased with increasing VCF. This may be justified by the fact that due to high concentration of solute molecules in feed side, concentration polarization provides the negative influence on permeate flux. It is also observed that activities of β-galactosidase is increased with increasing VCF.
In Fig. 11, time histories of permeate flux has been shown for 300 kDa membrane at different stirrer speed, ranging from 50 r.p.m. to 200 r.p.m. for providing the required turbulence in the membrane module to reduce the effects of concentration polarization. It is observed that permeate flux is increased gradually with increasing stirrer speed (50 rpm-150 rpm) but at 200 r.p.m. it becomes almost same with just lower one.

It is notified from Fig. 11 that rate of flux decline is higher with low stirrer speed. Permeate flux was found to increase with the increase of stirring because of enhanced turbulence at the membrane surface. The possible cause of higher flux and low rate of flux declination is that when stirring, the feed adjacent to the membrane surface also rotates, and sweeps away the deposited solutes from the membrane surface resulting in a reduction of concentration polarization, accompanied by the increase of rate of permeation. It is also notified that permeate flux is declined rapidly, then gradually and eventually it becomes steady with time axis. Time history of permeate flux at different TMP during the first stage purification process is depicted in Fig. 12. In this case stirrer speed always maintained 150 rpm at constant way and 4 stages of DF was conducted at VCF 4.

In this case permeate flux was found to increase with the increase TMP (98.07 kPa cm$^{-2}$ to 294.2 kPa) and at TMP 392.27 kPa it becomes almost similar with lower one. This may be caused for low concentration polarization of solute molecules on the membrane surface. High TMP provides high shear on the membrane surface by which large molecular weight solutes are rejected rapidly. It is also notified that permeate flux is declined rapidly, then gradually and eventually it becomes steady with time axis. It has been observed that activities of β-galactosidase is increased with increasing TMP, ranging from 98.07 kPa to 294.2 kPa at constant stirrer speed (150 rpm) and VCF 4. Fig. 13 shows the variation of permeate flux as a function of time at different membrane rotation speed keeping all other parameters constant.
The decline of the flux with time is obvious for pressure driven membrane separation process like UF, which is attributed to the concentration polarization and fouling. The later is found to be reversible in nature in this case. The flux enhancement with the increase in the membrane rotation speed can be observed from the figure, which resulted from a reduced concentration polarization for a rotating membrane disc. For the lower membrane rotation speed, the concentration polarization and the resulting fouling become very severe as the UF process goes on, which reduce the flux drastically. It is observed that activity of β-galactosidase is increased with increasing membrane rotation speed. Comparing Fig. 11, Fig. 12 and Fig. 13 it may be say that membrane rotation is more influencing parameter compare to stirrer speed and TMP, with the aid of fine protein purification as well as high permeate flux. From this investigation, it is clear that optimum stirrer speed, TMP and VCF and membrane rotation speed in UF process are 150 r.p.m., 294.2 kPa, 4 and 80 r.p.m. respectively. At optimum condition of UF technique 45 fold β-galactosidase is purified with respect to crude extract and overall yield has been obtained 35%. Retentate of 300 kDa membrane at optimum condition was considered for isolating β-galactosidase by salting out method. Ammonium sulphate was added gradually into partially purified β-galactosidase at constant stirring condition. Target enzyme pellet was dialyzed for 24 hours by 1.0 kD dialysis membrane with reference of sodium phosphate buffer (pH 7). Finally, 55 fold β-galactosidase is purified with respect to crude extract and overall yield has been obtained 25%. Purified β-galactosidase was characterized with respect to maximum reaction velocity (vmax), Michelis Menten constant (Km), optimum reaction temperature and pH considering ONPG and lactose as a substrate. The values of maximum reaction velocity (vmax), Michelis Menten constant (Km) are found 12.65 μmole. mg protein⁻¹. min⁻¹ and 20.22 mM considering ONPG as a substrate and those are 24 μmole. mg protein⁻¹. min⁻¹, 20 mM respectively considering lactose as a substrate. In Fig. 14, enzyme activities are plotted against reaction temperature considering ONPG as a substrate. In Fig. 15, enzyme activities are plotted against reaction pH considering ONPG as a substrate.

In the present investigation production of intracellular β-galactosidase has been performed by isolated facultative anaerobic consortium Bacillus safensis (JUCHE1) in the lab scale fermentor by batch mode. Composition and pH of microbial growth medium, incubation temperature, stirrer speed were varied to assess the microbial growth and β-galactosidase production. It is observed that microbial growth is high in MMRS medium compare with lactose broth. Optimum lactose concentration in MMRS medium is found 20 g.L⁻¹. In the present investigation, it is found that tryptone, commonly known as casein hydrolysate has the best influence on microbial growth compare with other two nitrogen sources such as peptone and casein. Optimum stirrer speed, incubation temperature and pH of microbial growth medium are found 150 r.p.m., 37°C and 7 respectively. In the present investigation sonication was adopted to isolate intracellular β-galactosidase. Optimum sonication time is found 300 seconds with inter stage cooling for 10 seconds after an operating time of 30 seconds. In UF technique different operating parameters, such as, TMP (98.07 kPa to 392.27 kPa), stirrer speed, and the feed flow rate were varied to assess the performance of UF process. It is observed that optimum permeate flux is obtained at 300 kDa membrane and TMP of 294.2 kPa. In the present investigation sonication was adopted to isolate intracellular β-galactosidase. Optimum sonication time is found 300 seconds with inter stage cooling for 10 seconds after an operating time of 30 seconds. In UF technique different operating parameters, such as, TMP (98.07 kPa to 392.27 kPa), stirrer speed, and the feed flow rate were varied to assess the performance of UF process. It is observed that optimum permeate flux is obtained at 300 kDa membrane and TMP of 294.2 kPa.

IV. CONCLUSION

In the present investigation production of intracellular β-galactosidase has been performed by isolated facultative anaerobic consortium Bacillus safensis (JUCHE1) in the lab scale fermentor by batch mode. Composition and pH of microbial growth medium, incubation temperature, stirrer speed were varied to assess the microbial growth and β-galactosidase production. It is observed that microbial growth is high in MMRS medium compare with lactose broth. Optimum lactose concentration in MMRS medium is found 20 g.L⁻¹. In the present investigation, it is found that tryptone, commonly known as casein hydrolysate has the best influence on microbial growth compare with other two nitrogen sources such as peptone and casein. Optimum stirrer speed, incubation temperature and pH of microbial growth medium are found 150 r.p.m., 37°C and 7 respectively. In the present investigation sonication was adopted to isolate intracellular β-galactosidase. Optimum sonication time is found 300 seconds with inter stage cooling for 10 seconds after an operating time of 30 seconds. In UF technique different operating parameters, such as, TMP (98.07 kPa to 392.27 kPa), stirrer speed, and the feed flow rate were varied to assess the performance of UF process. It is observed that optimum permeate flux is obtained at 300 kDa membrane and TMP of 294.2 kPa.
speed (50 r.p.m. to 200 r.p.m.), membrane rotation speed (40 r.p.m. to 100 r.p.m.) and VCF (2 to 4) were varied. Under the present investigation, four stage DF were carried out for purification of β-galactosidase. It was observed that membrane rotation has much more efficient than stirrer speed and TMP for diminishing the concentration polarization. Optimum stirrer speed, TMP, VCF and membrane rotation were found 150 r.p.m., 294.2 kPa, 3 and 80 r.p.m. respectively. Finally, 55 fold β-galactosidase is purified with respect to crude extract and overall yield has been obtained 25%. The values of maximum reaction velocity ($v_{\text{max}}$), Michelis Menten constant (Km) are found 12.65 µmole. mg protein$^{-1}$, min$^{-1}$ and 20.22 mM considering ONPG as a substrate and those are 24 µmole. mg protein$^{-1}$. min$^{-1}$, 20 mM respectively considering lactose as a substrate. Optimum temperature and pH of enzymatic reaction are found 45°C and 7 respectively.

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REFERENCE


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