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Ethanol production from deproteinized cheese whey powder in a batch fermentation process: optimization of process and kinetic modelling

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ABSTRACT

Cheese whey powder (CWP) is an interesting raw material for bioethanol production, since it is a dried and concentrated form of cheese whey, a dairy wastewater and contains high lactose content along with other nutrients. In order to reduce the production cost and to minimize the harmful effect on the environment, it is advantageous to study the treatment of wastewater containing carbohydrates for use as substrates for ethanol production. In the present study, deproteinized CWP solution was used as fermentation medium for ethanol production using *Kluyveromyces marxianus* strain NCIM 3217 in batch experiments. The physical parameters of fermentation such as temperature and pH were optimized and were found to be 35°C and 4.5, respectively, for the highest yield of ethanol for 24 h. At the optimized conditions of temperature and pH, the effect of initial sugar concentration (between 150 and 250 g/L) of feed CWP solution was studied for 72 h. Maximum ethanol production of 59.33 g/L was achieved at initial lactose concentration of 200 g/L, and therefore, it could be used as the most appropriate substrate dosage with which optimal production of ethanol and high substrate utilization could be obtained. Above 200 g/L lactose concentration, both biomass and ethanol concentrations were decreased since the hypertonic condition caused by high levels of substrate might has abated the viability and fermentation ability of the yeast. Ethanol fermentation was modelled using unstructured, kinetic models under optimized conditions of temperature and pH to depict the importance of yeast growth, product formation and substrate utilization for all the three lactose concentrations. Monod and Leudeking–Piret equations were used for batch fermentation with regard to lactose utilization and incubation time. The obtained results showed an acceptable fitting of the experimental data to the kinetic models with high significant R^2 values and, therefore, may be applied for the production of ethanol by fermentation of CWP. Biomass yield ($Y_{X/S}$), product yield ($Y_{P/S}$) and ethanol productivity (Q_p) were all found to be highest at 200 g/L lactose.

Keywords: Cheese whey powder; Ethanol; Lactose fermentation; Growth kinetics; *Kluyveromyces marxianus*

1. Introduction

Production of biofuel from microbial sources using waste by-products as substrates has gained much considerable importance in the recent years in order to fulfil world's energy demand and to reduce air pollution. Bioethanol and other biofuels offer advantages over fossil fuels since it provides renewable and sustainable energy sources and can be used as a cheap and clean alternative fuel source, fuel additive and gasoline enhancer [1]. Among various raw materials used for bioethanol production, whey, a by-product of dairy industries, can be used as substrate, which is inexpensive and highly available. Cheese/casein whey represents an important source of environmental pollution because of the high

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production rate all over the world and its high organic matter content with biochemical oxygen demand (BOD) ranging from 27 to 60 g/L and chemical oxygen demand (COD) ranging from 50 to 102 g/L [2]. The major components of cheese whey are lactose (5%–6% w/v), protein (0.8%–1.0%), fat (0.06%) and mineral salts. Among various components of whey, lactose content is mainly responsible for its high BOD [3]. Hence, production of ethanol from whey has gained importance since it would not only be used to meet the global fuel demand but would also help in reducing the environmental pollution leading to whey bioremediation. Even though cheese whey is an inexpensive and an easily available raw material, fermentation of non-concentrated cheese whey for production of ethanol is not economically feasible as the concentration of ethanol obtained at the end of fermentation process has been found to be very low (only about 2%), thus making the distillation process for separation of ethanol too expensive [4]. Ultrafiltration processes have been used to improve the lactose concentration of cheese whey, but the process is expensive (~50 USD/m^3) and can help increase the lactose concentration by a factor of 5 only [5]. However, when concentrated whey permeate is used instead of unconcentrated whey permeate, production of ethanol is reportedly higher; making it a likely choice for an optimized ethanol production process, from whey [6]. Utilization of cheese whey powder (CWP), which is a dried and concentrated form of cheese whey, for ethanol fermentation, has significant advantages such as elimination of costly ultrafiltration processes, compact volume, easy transport, long-term stability and high concentration of lactose and other nutrients (nitrogen and phosphate) yielding high ethanol concentrations, thus making the fermentation process economically more feasible [7,8]. Moreover, the cost of production of CWP from cheese whey is much less than the distillation cost for ethanol separation from dilute casein whey [5]. *Kluyveromyces* strains are the most widely used yeast strains in fermentation process and are known to produce ethanol directly from lactose present in cheese whey due to the presence of galactose fermenting capability [9].

To effectively analyze and optimize a biological process, the kinetics of the process is required to be studied. In the present work, ethanol production employing *Kluyveromyces marxianus* using CWP solutions was studied with an objective to optimize the physical parameters of fermentation such as temperature and pH, investigate the effect of various initial lactose concentrations (150, 200 and 250 g/L) on ethanol production, and analyze the kinetic and stoichiometric parameters (yield coefficients) under optimized temperature and pH conditions for better understanding of ethanol fermentation process. The kinetic model parameters, biomass yield, product yield and ethanol productivity were estimated using the experimental data.

2. Materials and methods

2.1. Chemicals

CWP was purchased from Alpha Overseas, New Delhi, India. Pure lactose was supplied by Loba Chemie, Mumbai, India, and was used for the preparation of lactose standard curve to be used during the analysis of sugar consumption during fermentation. Malt extract, yeast extract, peptone and agar were purchased from Hi Media Chemicals, Mumbai, India. Ammonium sulphate and potassium dihydrogen orthophosphate were purchased from SRL, Mumbai, India. Glucose, phenol and sulphuric acid were obtained from Merck Limited, Mumbai, India.

2.2. Microorganism and maintenance

For alcoholic fermentation process, a strain of the yeast *K. marxianus* was used. The production of ethanol and other intermediate metabolites (pyruvic acid, citric acid, acetic acid, etc.) is reportedly increased in the presence of higher concentrations of initial lactose, during fermentation of whey by *K. marxianus*, due to a switch in its metabolic pathways (from an oxidative pathway to a mixed oxidative pathway) [10]. Strain NCIM 3217 was procured from the culture collection of the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, Maharashtra, India. The yeast was maintained on agar plates having the following composition (g/L): glucose, 10; malt extract, 3; yeast extract, 5; peptone, 5; and agar, 20, and pH adjusted to 6.4–6.8. A 24-h growth was preserved at a temperature of 4°C for further use.

2.3. Yeast culture preparation

The inoculum was prepared by transferring a loopful of cells of *K. marxianus* NCIM 3217 from a freshly grown culture to 250 mL Erlenmeyer flasks containing 100 mL sterile CWP solution (50 g/L lactose) supplemented with other media components (malt extract, yeast extract and peptone) and plugged in with cotton. The other media components used for growth of yeasts were given in the same ratio as was used for agar plates. The medium was sterilized at 121°C for 20 min. The flask was incubated in an orbital shaker at 100 rpm for 24 h at 30°C.

2.4. Preparation of fermentation medium for ethanol production

The CWP contain more than 70% lactose and also have proteins, fat, ash and moisture. To be used for fermentation medium, CWP solution was diluted with deionized water to prepare different concentrations of lactose solutions (150, 200 and 250 g/L) and was also supplemented with the following media components in g/L: malt extract, 3; yeast extract, 3; peptone, 5; ammonium sulphate, 2; and potassium dihydrogen orthophosphate, 1. Feed CWP solution was deproteinized by heat treatment at 115°C for 15 min [11]. The precipitates were removed by centrifugation at 11,000 rpm (13,000 × g) for 15 min at 10°C, and the supernatants were sterilized by autoclaving at 121°C for 20 min and then used for fermentation medium.

2.5. Experimental system

Batch fermentations were carried out in an orbital shaker using sterile Erlenmeyer flasks sealed with cotton plugs so as to reduce oxygen permeability. The fermentation media were inoculated with 10% (v/v) yeast inoculum. Temperature and pH were optimized and were used for studying the effect of initial sugar concentrations and kinetic modelling. Samples

were collected at the beginning of fermentation (time = 0 h) and subsequently at time t = 20, 24, 44, 48, 68 and 72 h from fermentations flasks aseptically to determine cell growth, lactose consumption and ethanol production. The incubation period was for 72 h. Each experiment was replicated thrice to check the reproducibility of the results.

2.6. Effect of temperature and pH

The effect of temperature on ethanol production was studied by carrying out fermentation experiments for 24 h at different temperatures (25°C, 30°C, 35°C and 40°C) using CWP solutions, keeping initial lactose concentration at 200 g/L, pH 4.5 and inoculum size 10% (v/v).

Similarly, the effect of pH on ethanol production was studied by carrying out fermentation experiments for 24 h at different pH (3.5, 4.5, 5.5 and 6.5) using CWP solutions, keeping initial lactose concentration at 200 g/L, temperature 35°C and inoculum size 10% (v/v). The optimized temperature and pH were used for studying the effect of initial lactose concentrations and for calculating the kinetic parameters of fermentation.

2.7. Analytical methods

2.7.1. Determination of ethanol and sugar concentration

The fermentation media was centrifuged at 11,000 rpm (13,000 × g) for 15 min, and the supernatant was used for lactose and ethanol quantification. Ethanol concentrations were measured using a gas chromatograph (Varian CP-3800) with a flame ionization detector and a wall-coated open-tubular fused silica capillary column (15 m × 0.25 mm internal diameter, 0.25 μm film thickness). The column temperature was set at 75°C for 1 min and raised to 130°C with a rate of 20°C/min yielding a total holding time of 4.75 min. Temperatures of injector and detector were 150°C and 200°C, respectively. Nitrogen was used as the carrier gas with a velocity of 25 mL min^{-1}. Ethanol was quantified by means of a calibration (peak area vs. concentration) performed before actual samples were injected. Each sample was injected three times to assure reproducibility.

Lactose concentration was estimated by taking the absorbance of the supernatant in a UV-Vis spectrophotometer (Varian Cary 50Bio, Part No. EL07113760) at 490 nm by the phenol–sulphuric acid method [12].

2.7.2. Estimation of biomass concentration

Biomass was measured in terms of dry weight. Yeast cells were harvested by centrifugation for 10 min at 11,000 rpm (13,000 × g) and then washed twice with distilled water and weighed after 24 h at 100°C.

2.8. Kinetic modelling of fermentation

Kinetic models predict how fast the microorganisms can grow and use substrates or make products. Kinetic data are needed to develop basic understanding of fermentation processes and can be very useful for the design of both continuous and batch production systems. Various structured and unstructured models are available for describing fermentation processes. Unstructured, non-segregated kinetic models play an important role in monitoring batch fermentation process and are much easier to use, and have been applied for the description of a wide range of experimental conditions and media [13].

Rate equations for biomass, ethanol production and lactose consumption were used to describe the fermentation process.

2.8.1. Kinetic study of microbial growth

The most commonly applied unstructured, non-segregated model of microbial growth is the Monod equation, and it empirically fits a wide range of data satisfactorily [13]. The biomass growth rate (dX/dt) can be described as follows:

$$\frac{dX}{dt} = \mu X \tag{1}$$

where the specific growth rate μ is given by the Monod equation, and X is the biomass concentration.

The Monod equation expresses the relationship between μ and the residual growth limiting substrate, and is given below:

$$\mu = \mu_{max}\left(\frac{S}{K_s + S}\right) \tag{2}$$

where μ is the specific growth rate; μ_{max} is the maximum specific growth rate constant for the organism; and S is the substrate concentration. K_s is the saturation constant and is defined as the substrate concentration when $\mu = \mu_{max}/2$, and it represents an affinity of the organism for the nutrient. The kinetic parameters were the Monod parameters (K_S and μ_{max}) and were dependent on the organism, the growth limiting nutrient, fermentation medium and environmental factors such as pH and temperature. This model (Eq. (2)) expresses that the specific growth rate of microorganisms decreases if the substrate concentration is decreased and vice versa. K_S and μ_{max} were determined from the double-reciprocal form of the Monod equation known as Lineweaver–Burk plot, which is given below:

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}}\frac{1}{S} \tag{3}$$

2.8.2. Kinetic study of product formation

The Leudeking–Piret equation describes the mixed growth-associated product formation model in the fermentation process [14]. The product formation rate (dP/dt) depends both on instantaneous biomass concentration (X) as well as growth rate dX/dt in a linear fashion:

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \tag{4}$$

where α and β are the two estimated parameters for kinetic expression that may vary with fermentation conditions. A plot of $1/X(dP/dt)$ vs. $1/X(dX/dt)$ was found to be linear with a slope of α, and an intercept of β. The values of these parameters have been determined and reported.

2.8.3. Kinetic study of substrate utilization

The substrate utilization kinetics for ethanol fermentation can be expressed by Eq. (5), which considers that a carbon substrate such as lactose is consumed for the maintenance of the cell as well as for the formation of cell material and metabolic products [15]. The equation is expressed as follows:

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} - m_S X \tag{5}$$

where dS/dt is the substrate utilization rate; $Y_{X/S}$ and $Y_{P/S}$ are the yield coefficients for the biomass and product, respectively; and m_S is the specific maintenance coefficient. Substituting Eq. (4) into Eq. (5), the substrate material balance can be rewritten as follows:

$$\frac{dS}{dt} = \left[\frac{1}{Y_{x/s}} + \frac{\alpha}{Y_{p/s}}\right]\frac{dX}{dt} + \left[\frac{\beta}{Y_{p/s}} + m_S\right]X \tag{6}$$

$$\frac{dS}{dt} = -\gamma\frac{dX}{dt} - \delta X \tag{7}$$

where $\gamma = \left[\frac{1}{Y_{x/s}} + \frac{\alpha}{Y_{p/s}}\right]$ and $\delta = \left[\frac{\beta}{Y_{p/s}} + m_S\right]$; γ and δ are parameters for growth and non-growth-associated substrate consumption, respectively. The values of these parameters may vary with fermentation conditions. A plot of $1/X(dS/dt)$ vs. $1/X(dX/dt)$ was found to be linear with slope γ, and intercept δ, which have been reported.

2.9. Stoichiometric parameters for fermentation

Kinetic models predict how fast the microorganisms can grow and use the substrates to produce products whereas stoichiometric models predict how much substrate is needed or product is produced for a known amount of biomass, or vice versa. Thus, stoichiometric parameters (biomass yield and product yield) are also important parameters to be analyzed for monitoring the fermentation process.

Biomass yield ($Y_{X/S}$, g cells/g substrate) was defined as the ratio of the weight of biomass produced per weight of substrate utilized and is given by:

$$Y_{X/S} = -\frac{X - X_0}{S - S_0} \tag{8}$$

where X_0 and X are initial and final biomass concentration (g/L), and S_0 and S are initial and final substrate concentration (g/L), respectively.

Product yield ($Y_{P/S}$, g product/g substrate) was defined as the weight of product produced per weight of substrate utilized and is given by:

$$Y_{P/S} = -\frac{P - P_0}{S - S_0} \tag{9}$$

where P_0 and P are initial and final product concentration (g/L), respectively.

Ethanol productivity (Q_p, g/L-h) is also an important parameter and is defined as the ratio between ethanol concentration (g/L) and fermentation time (hours) and is given by:

$$Q_P = \frac{E - E_0}{h} \tag{10}$$

where E_0 and E are initial and final ethanol concentration (g/L), respectively, and hour (h) is the fermentation time.

3. Results and discussion

3.1. Effect of temperature and pH

To study the effect of temperature (25°C, 30°C, 35°C and 40°C) on ethanol production from CWP with initial substrate concentration of 200 g/L, fermentation was carried out for a period of 24 h. The results have been shown in Fig. 1. As the temperature was increased from 25°C, ethanol production was found to first increase, and a maximum ethanol production of 19.96 g/L was found corresponding to temperature 35°C. Ethanol production was found to decrease with further increase in temperature. This might be due to the fact that above 35°C temperature, the growth rate decreased and thermal death might have occur, effecting the ethanol production. Also, when the temperature was increased above 35°C, the maintenance requirements of the cells increased leading to higher substrate consumption for cellular maintenance, which represents energy expenditures to repair damaged cellular components, to transfer some nutrients and product in and out of cells and to adjust the osmolarity of the cells' interior volume. As the temperature was increased in the range of 25°C–35°C, the activity of the microorganisms favouring the consumption of lactose increased and thus had a positive effect on ethanol production. Thus, the optimum temperature was found to be 35°C.

Similarly, to study the effect of pH (3.5, 4.5, 5.5 and 6.5) on ethanol production from CWP solutions with initial substrate concentration of 200 g/L, fermentation was carried out for 24 h. The results have been shown in Fig. 2. From

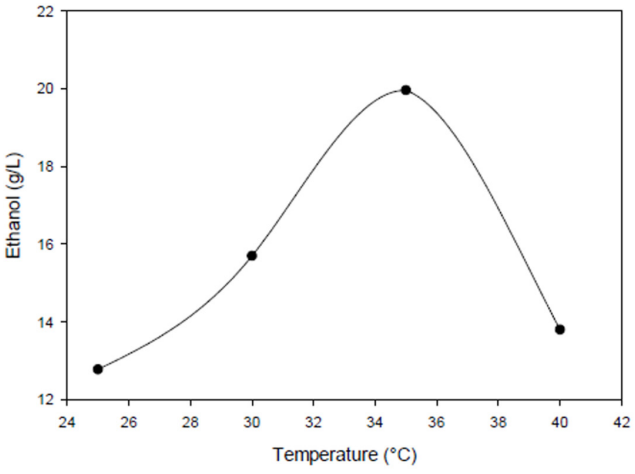

Fig. 1. Effect of temperature on ethanol production using *K. marxianus*, pH 4.5, and inoculum size 10% (v/v).

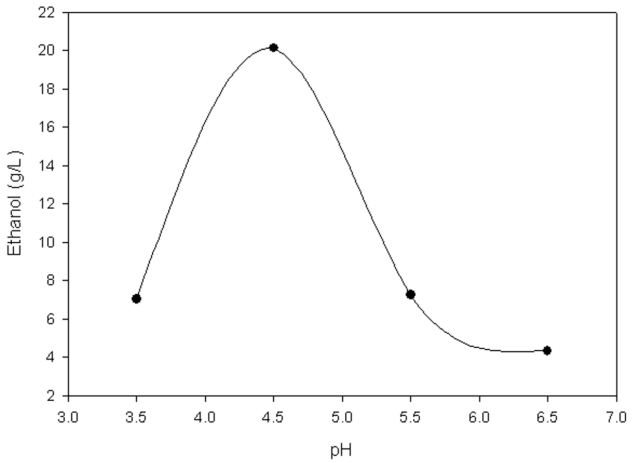

Fig. 2. Effect of pH on ethanol production using *K. Marxianus*, temperature 35°C, inoculum size 10% (v/v).

Fig. 2, it can be observed that when pH was increased from 3.5 to 4.5, ethanol production was increased but it decreased when pH was further increased above 4.5. Maximum ethanol production of 20.12 g/L was found at pH 4.5. This might be due to the fact that above pH 4.5, the maintenance energy requirements of the *K. marxianus* yeast cells increased; thus, utilization of lactose to produce ethanol decreased. Thus, the optimum pH was found to be 4.5. These optimum temperature and pH were used for further studying the effect of different lactose concentrations and kinetic modelling of the fermentation process.

3.2. Microbial growth kinetics

Monod equation defines the biomass growth rate as a rational relationship. The Monod kinetic model was plotted as a double reciprocal graph (Eq. (3)) based on experimental data for substrate consumption by the yeast and incubation time. For different initial lactose concentrations, the kinetic parameters were estimated and reported in Table 1. For 150 g/L lactose concentration, the values of kinetic parameters (μ_{max} and K_S) obtained from the parametric estimation were 0.0213 h^{-1} and 23.732 g/L, respectively (Table 1). Values of maximum specific growth rate (μ_{max} = 0.0254 h^{-1}) and saturation constant (K_S = 30.671 g/L) were found to be highest for 200 g/L initial lactose concentration. For all the three cases, the correlation coefficient, R^2, values were close to 1 (0.9464 for 150 g/L; 0.9136 for 200 g/L and 0.9 for 250 g/L), as shown in Fig. 3. Thus, it may be stated that the proposed Monod model was adequate to explain the growth profile of the yeast during batch fermentation of ethanol from CWP solutions.

3.3. Product formation kinetics

From the experimental study, it was observed that ethanol production followed Leudeking–Piret model, and the kinetic parameters (α and β) were evaluated by Eq. (4) for different lactose concentrations (150, 200 and 250 g/L) and were reported in Table 1. According to the Luedeking–Piret

Table 1
Values of kinetic model parameters for growth kinetics, product formation and substrate consumption

Kinetic models	Substrate (lactose) concentration			
		150 g/L lactose	200 g/L lactose	250 g/L lactose
	Growth kinetics			
Monod model	μ_{max}, h^{-1}	0.0213	0.0254	0.0139
	K_S, g/L	23.732	30.671	27.07
	Product formation kinetics			
Leudeking–Piret model	α	6.9816	5.3628	10.418
	β	0.0715	0.1058	0.0805
	Substrate consumption kinetics			
	γ	16.227	16.361	40.473
	δ	0.2697	0.2085	0.32

Eq. (4), α is the growth-associated constant and β the non-growth-associated constant. When α is zero, the product is non-growth associated, and when β is zero, the product is only growth associated. For 150 g/L lactose concentration, the experimental values of α and β were obtained from the slope and intercept of the graph plotted for Eq. (4) (Fig. 4(A)) as 6.9816 and 0.0715, respectively. The results showed that the value of growth-associated constant 'α' is much greater than the non-growth-associated rate constant 'β'. Hence, the ethanol production in this study was observed as growth-associated product formation. The experimental data were fitted with this model, and the R^2 values for all the three lactose concentrations were found to be close to 1 (0.9937, 0.9779, 0.9978), as shown in Fig. 4.

3.4. Substrate utilization kinetics

Leudeking–Piret model was used for the study of substrate consumption kinetics. Lactose utilization kinetics was represented by Eq. (7). The kinetic parameters, γ and δ, for all the three lactose concentrations have been estimated and reported in Table 1. For lactose concentration 150 g/L, a plot of Eq. (7), shown in Fig. 5(A), yielded the kinetic parameters as γ = 16.227 (growth associated) and δ = 0.2697 (non-growth associated) from the slope and intercept, respectively. When the experimental data were fitted to the model, it was found that the R^2 values for all the lactose concentrations were close to 1 (0.9836, 0.9907, 0.9977), depicted in Fig. 5. Thus, it can be said that the substrate utilization kinetics during fermentation of CWP solutions followed the proposed Leudeking–Piret model.

3.5. Effect of initial substrate concentration

The effect of initial substrate concentrations on ethanol production by *K. marxianus* NCIM 3217 was studied using lactose as a substrate by carrying out the experiments at different initial substrate concentrations (150, 200 and 250 g/L) keeping the temperature at 35°C, pH at 4.5 and inoculum size of 10% (v/v). An incubation time of 72 h was considered in all the fermentation processes. Fig. 6(A) shows the kinetics

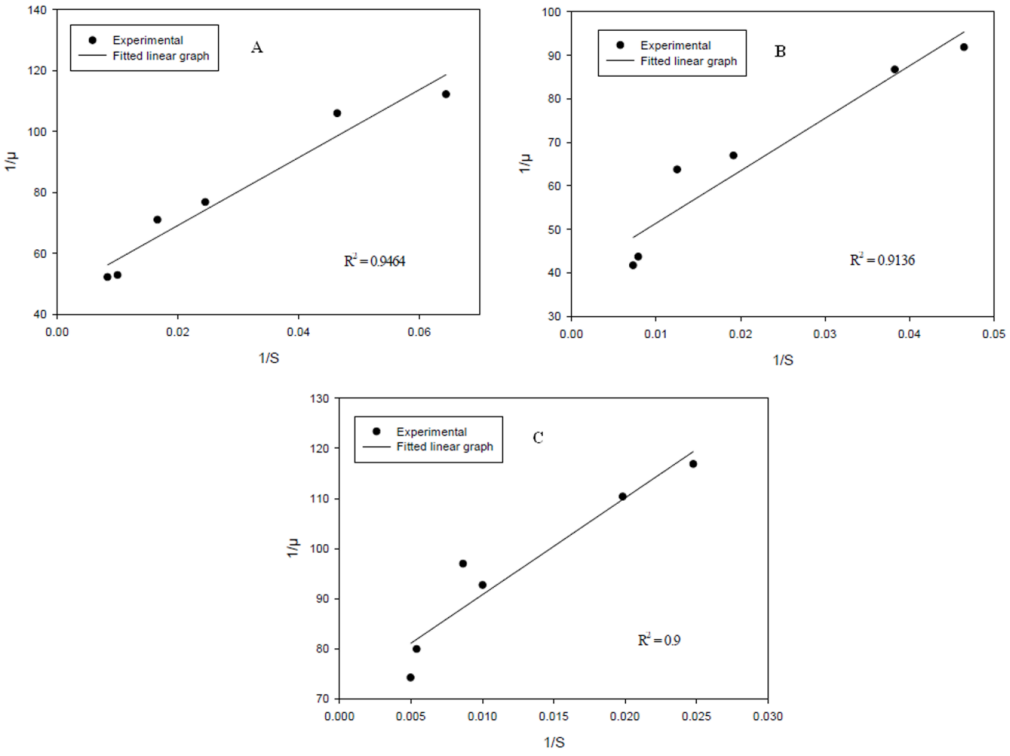

Fig. 3. Experimental data fitted to the Monod kinetic model at pH 4.5 and temperature 35°C: (A) at 150 g/L lactose; (B) at 200 g/L lactose; and (C) at 250 g/L lactose.

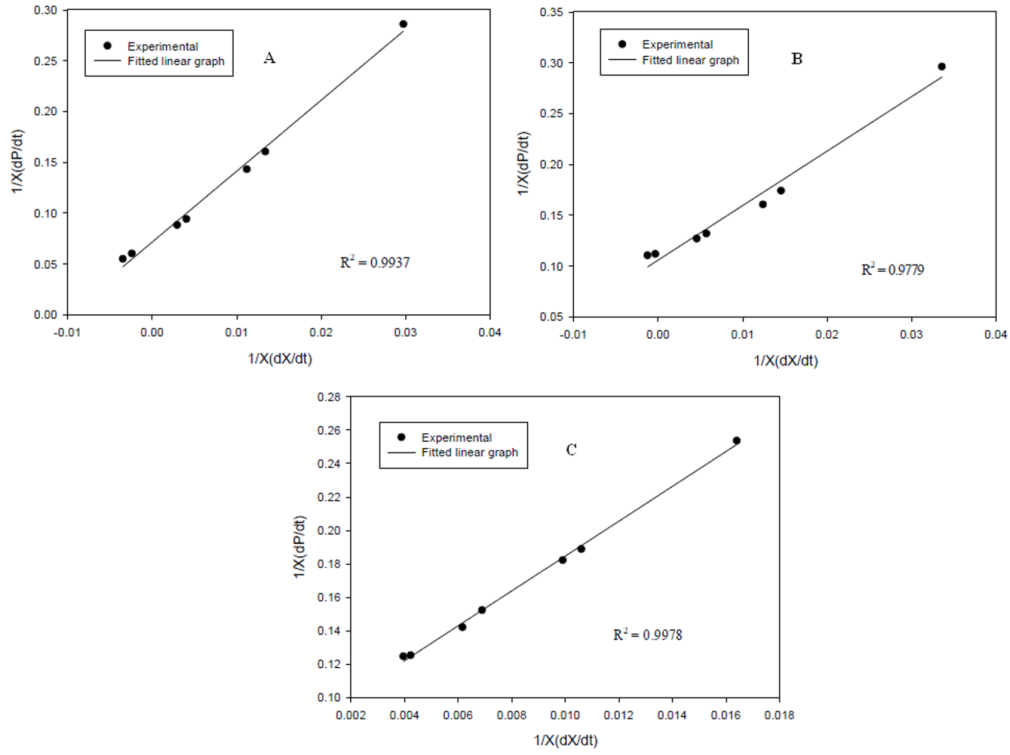

Fig. 4. Experimental data fitted to the Leudeking–Piret model for product formation at pH 4.5 and temperature 35°C: (A) at 150 g/L lactose; (B) at 200 g/L lactose; and (C) at 250 g/L lactose.

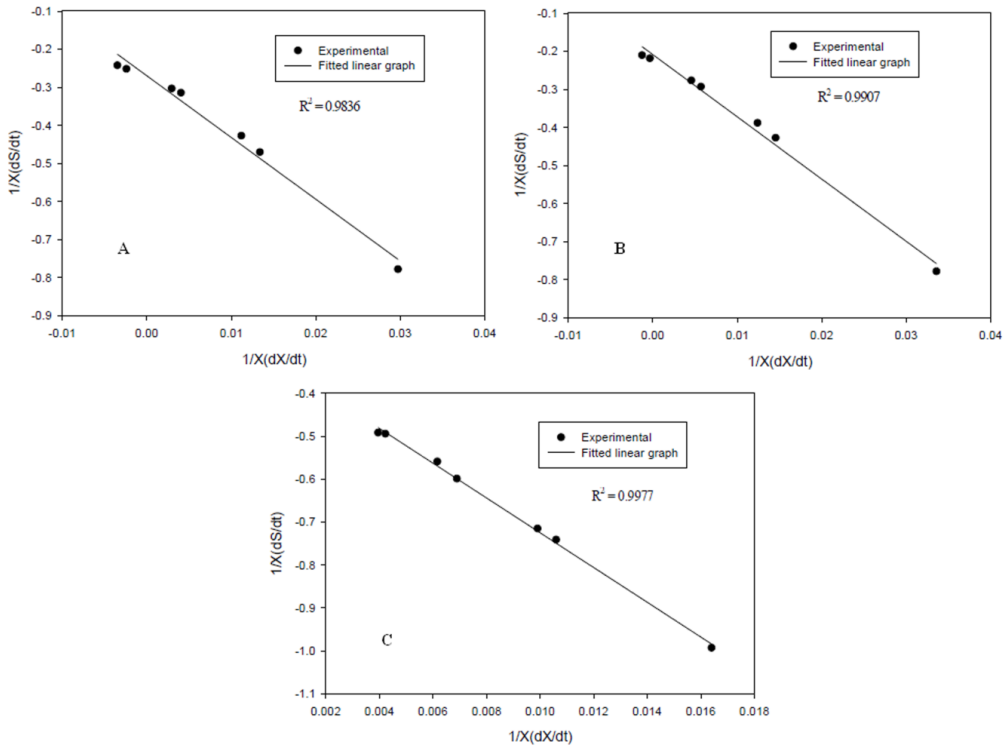

Fig. 5. Experimental data fitted to Leudeking–Piret model for substrate consumption at pH 4.5 and temperature 35°C: (A) at 150 g/L lactose; (B) at 200 g/L lactose; and (C) at 250 g/L lactose.

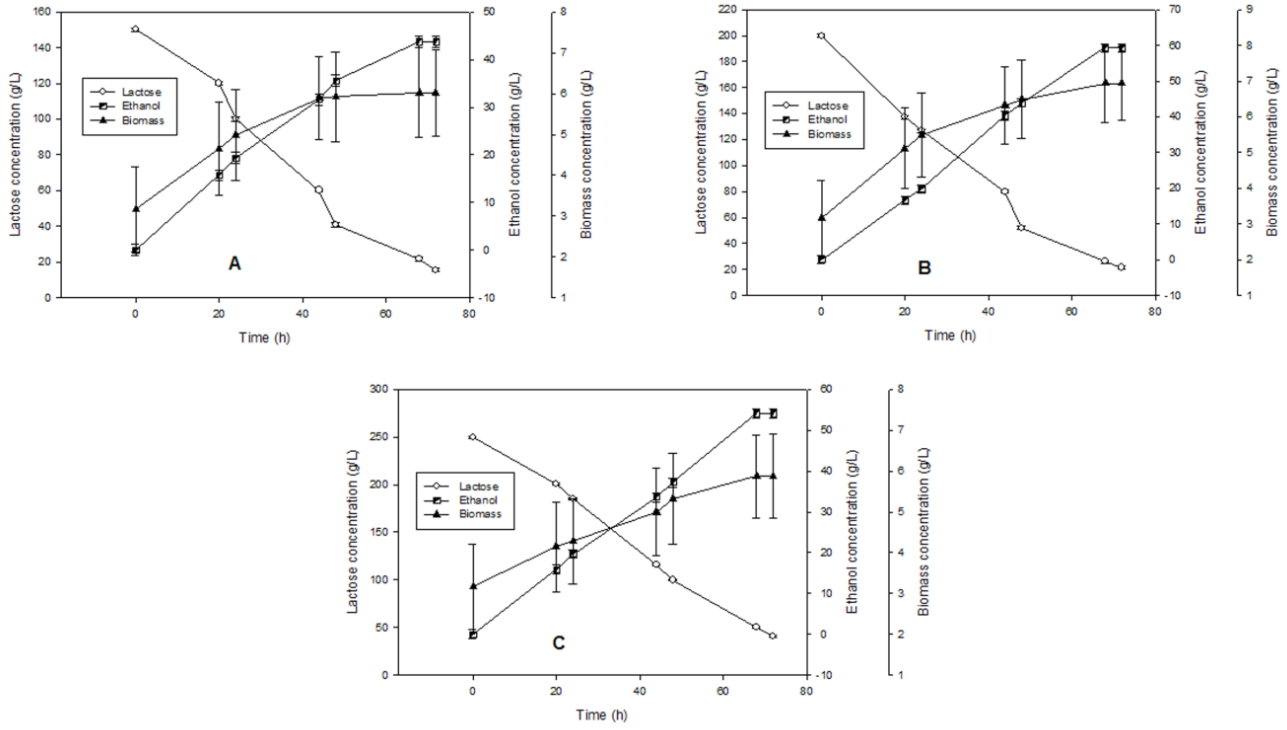

Fig. 6. Production of biomass and ethanol, and substrate utilization in lactose fermentation at pH 4.5 and temperature 35°C: (A) for lactose 150 g/L; (B) for lactose 200 g/L; (C) for lactose 250 g/L.

of batch fermentation of lactose to ethanol by *K. marxianus* NCIM 3217 at S_0 = 150 g/L. Lactose utilization started within 24 h, and most of the lactose was consumed in 72 h. *K. marxianus* could metabolize most of the lactose within 72 h giving ethanol concentration of 43.71 g/L and biomass concentration of 6.02 g/L. It can be observed from the graph (Fig. 6(A)) that after 68th h, ethanol and biomass concentration became constant.

When deproteinized CWP solution containing 200 g/L lactose was fermented, ethanol and biomass concentration increased to 59.33 and 6.94 g/L, respectively, at the end of 72 h, evident from the time profile graph (Fig. 6(B)). This growth pattern for the yeast was observed because lactose is an important signal molecule for the yeast, apart from being a carbon source. Thus, the yeast grew better as a result of increased availability of sugar in their environment and produced higher concentrations of ethanol. When initial lactose concentration of CWP solution was further increased to 250 g/L, there was a decrease in ethanol and biomass production as seen in the graph (Fig. 6(C)). The ethanol produced was 54.05 g/L, and the biomass produced was 5.87 g/L. This decrease in ethanol production with increase in initial lactose concentration may be due to the negative effect of the substrate that might have inactivated the yeast cells due to high osmotic pressure created at high sugar level, causing high maintenance requirements [5].

The hypertonic solution containing very highly concentrated substrate solution might have caused damage on membrane fluidity and intracellular enzyme activity, thus restraining the yeast growth and reducing the rate of ethanol fermentation. Severe decrease in membrane fluidity due to long-term exposure to hypertonic conditions makes it difficult for the substrate to enter and for the product to exit the cell, and as a result, the build up of ethanol and other toxic by-products in the cells might have caused biological damage to the yeast cell such as the transport and metabolic systems of the yeast [16]. Therefore, additional sugar sources were consumed by the yeast to maintain the activity of the transport system of essential materials under the stressed condition instead of being fermented to the final product ethanol.

Thus, it can be said that among three different initial substrate concentrations studied, lactose concentration of 200 g/L is the most favourable concentration for bioconversion to ethanol. Lactose concentration lower than 200 g/L would result in reduced ethanol production due to decreased sugar availability, and concentration higher than 200 g/L would no longer increase the ethanol production because of strong substrate inhibition along with product inhibition of the enzymes responsible for lactose conversion to ethanol.

3.6. Determination and comparison of stoichiometric parameters at different initial substrate concentration

Stoichiometric parameters such as biomass yield ($Y_{X/S}$), product yield ($Y_{P/S}$) and ethanol productivity (Q_p) have been found to vary with change in initial lactose concentration of CWP solutions, and the comparative results have been depicted in Fig. 7. Biomass yield ($Y_{X/S}$) was found to be similar up to 200 g/L lactose concentration, and then it decreased for 250 g/L. This might be due to the fact under the severely stressed fermentation conditions produced by high substrate

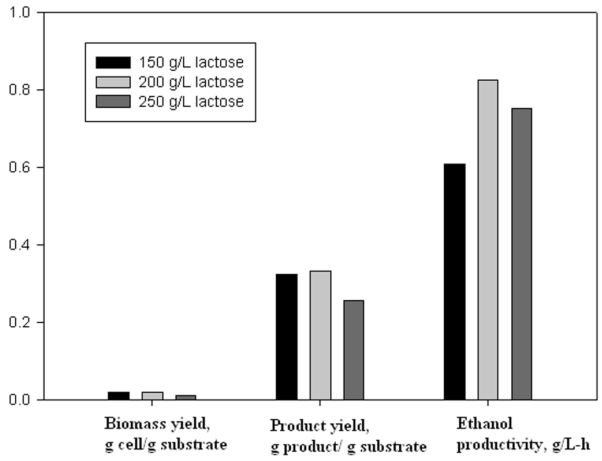

Fig. 7. Comparison of biomass yield ($Y_{X/S}$), product yield ($Y_{P/S}$) and ethanol productivity (Q_p) for different initial substrate concentrations at pH 4.5, temperature 35°C and inoculum size 10% (v/v).

concentration, additional energy and carbon sources were used by the yeast for survival in the hypertonic environment, rather than being used for growth and fermentation. Product yield was observed to increase with increase in lactose concentration up to 200 g/L. However, it got decreased for 250 g/L lactose concentration. Thus, both biomass yield and product yield were highest for 200 g/L corresponding to values of 0.021(g cell/g lactose) and 0.332 (g ethanol/g lactose), respectively. Among three different lactose concentrations (150, 200 and 250 g/L) studied, highest ethanol productivity was achieved at 200 g/L lactose concentration. Ethanol productivity at 200 g/L was 0.824 g/L-h. Thus, from the analysis of these parameters, it may be stated that concentration of 200 g/L lactose is the optimum for achieving the best possible ethanol production.

4. Conclusion

CWP is a concentrated form of cheese whey and can be used for ethanol production in desired concentrations. Temperature and pH are also important factors affecting the fermentation process and were optimized at 35°C and 4.5, respectively. Effect of different initial lactose concentrations (150, 200 and 250 g/L) on ethanol production by *K. marxianus* NCIM 3217 was investigated in this study, and to better understand the fermentation process, kinetic parameters as well as stoichiometric parameters were also analyzed. The initial lactose concentration in deproteinized CWP solution exerted great influence on ethanol production by *K. marxianus* NCIM 3217, maximum product formation (59.33 g/L) being obtained with initial lactose concentration of 200 g/L at pH 4.5 and temperature 35°C. Thus, it can be said that 200 g/L was the critical substrate concentration above which the membrane fluidity decreased leading to reduced yeast growth and product formation. The models projected in this study describe the biomass, ethanol production and substrate consumption with fermentation time in a good manner. Growth pattern of the yeast follows the Monod model, and the kinetic parameters were determined. Ethanol production was represented by Leudeking–Piret model, and based on

the values of kinetic parameters, it was observed that ethanol production by *K. marxianus* NCIM 3217 from various lactose concentrations was growth associated. For all the models, significance of correlation (R^2) values was close to 1, which depicts that the proposed models fitted the experimental data very well and may be useful for controlling the growth, ethanol production and substrate utilization in large-scale fermentation using *K. marxianus* NCIM 3217. Biomass yield, product yield and ethanol productivity were all found to be highest for initial lactose concentration of 200 g/L.

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