International Journal of Chemical Engineering Research.
ISSN 0975-6442 Volume 9, Number 2 (2017), pp. 223-228

http://www.ripublication.com

Kinetic Study of Enzymatic Hydrolysis of Lactose in Whey

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Abstract

Dairy industry is one of the largest industries that generate significant quantity of waste stream of which casein/cheese whey is the most abundant. Major constituent of whey is lactose and it contributes very high value of BOD and COD. Effort has been taken to convert the lactose, a disaccharide to fermentable sugar through enzymatic hydrolysis using commercially available enzyme β-galactosidase. The bioethanol derived from hydrolyzed whey-lactose could thus be a cost effective and environment friendly solution for treating this highly polluting waste stream. In this work, the enzymatic hydrolysis of lactose in whey was studied at room temperature of 32^0C. The lactose concentration was estimated using DNSA (Dinitro salicylic acid) method. The concentration of the hydrolyzed product, glucose was measured using GOD-POD test. On the basis of the data analyzed, Michaelis-Menten kinetic model has been represented. The parameters were estimated using lineweaver-burk plot. The maximum rate of hydrolysis was found to be 4.38 L/mol/min. The catalytic efficiency of the enzyme for the reaction has also been determined and reported.

Keywords: Lactose, whey, enzymatic hydrolysis, kinetic study, Lineweaver-Burk plot

1. INTRODUCTION:

Whey, the liquid remaining after separation of casein/cheese and fat during milk coagulation, is the principal by-product of dairy industry. Every year more than 3.2 million tonnes of lactose, dissolved in whey, is accrued by the cheese production worldwide. Almost half of this amount is used for human and animal nutrition. The

rest is waste and it is very difficult to dispose off the rest as it would cause severe environmental pollution. Therefore, there is a need for investigation about further utilization possibilities of lactose from whey. One of these applications with a high technological and dietetic interest is the enzymatic hydrolysis of lactose, whose economic importance has been increasing ever since the 1960s[1].In this process, the disaccharide lactose is converted into two simple monsaccharides, glucose and galactose.

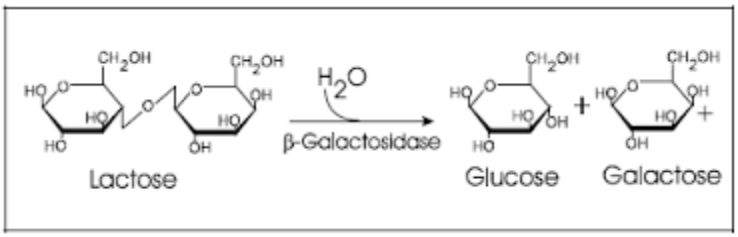

Fig.1 Conversion of lactose to glucose and galactose

Also, lactose present in milk or other dairy products cannot be digested by a large section of the human population creating various lactose intolerance symptoms. Therefore hydrolysis of lactose helps people to consume food products derived from milk/whey who suffer from lactose intolerance.

Hydrolyzed whey can be effectively converted to bio-ethanol with the help of the most popular microorganism involving fermentation, *Saccharomyces cerevisiae*; the bioethanol thus formed could be blended with gasoline for use in motor vehicle.

Two methods can be applied for lactose hydrolysis in whey and other dairy products: enzymatic hydrolysis and acidic hydrolysis. Enzymatic hydrolysis is preferable than acidic hydrolysis as the former process allows milder conditions of pH and temperature, and does not cause bad flavours, odors and colours. Furthermore, acidic method can cause protein denaturation which can be present in lactose solution and yield of undesirable by-products that could inhibit the hydrolysis[2,3].

In general, there are several technologies for enzymatic hydrolysis of lactose[4]. The easiest way is the discontinuous batch-process.

In this context, the present work has been undertaken with an objective to study the enzymatic hydrolysis of lactose solution in free enzyme mode. Attempt would be taken to find suitable kinetic model to represent the enzymatic hydrolysis reaction.

2. METHODOLOGY

In the laboratory, casein whey was prepared following iso-electric precipitation of casein protein of milk. The casein whey thus obtained has a pH of 4.8 and it was straw coloured. The lactose content of the whey thus formed was measured using DNSA (Dinitro salicylic acid) method[5] by measuring the absorbance of the solution at 540nm with the help of the calibration curve. Since lactose is a reducing sugar, it responds to the test wherein

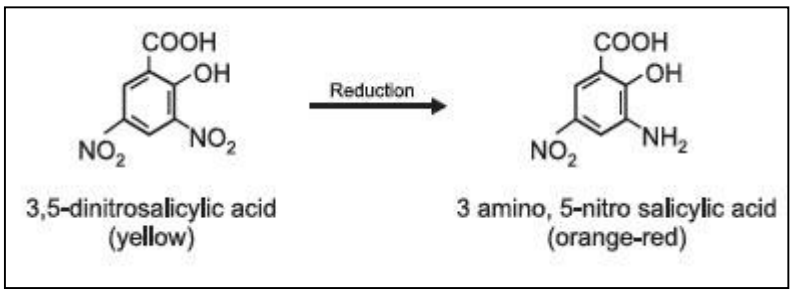

Fig.2: DNSA reaction

3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions. The lactose standard curve (Fig.3) was used to determine the concentration of lactose in whey.

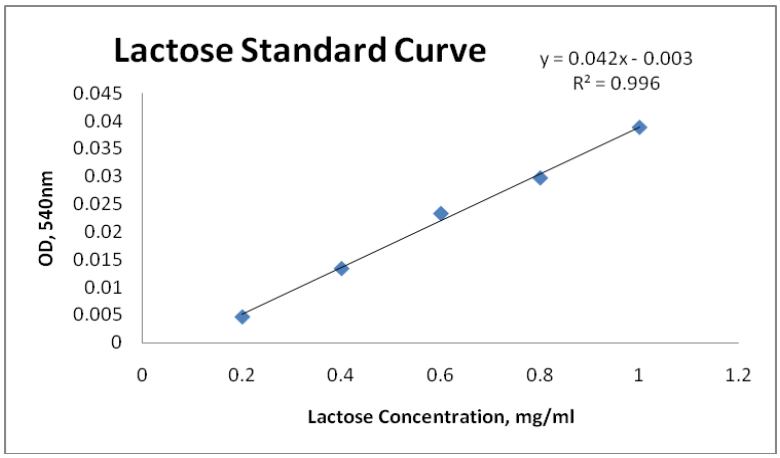

Fig 3: Lactose standard curve

To study the kinetics of lactose hydrolysis, 5 different lactose solutions (10, 20, 30,40, 50g/L i.e. 29 mM - 146 mM) were prepared separately on a buffer of 0.01 M K_2HPO_4, 0.015 M KCL and 0.012 M $MgCl_2.6H_2O$ at pH 6.75 adjusted with citric acid. 1 mL of the lactose sample was incubated with 1 mL of the enzyme solution (0.143 mmole /L) prepared on the same buffer for 10 minutes at room temperature of 32°C, and after

that 1 mL was extracted. The reaction was stopped by mixing with 1 mL of 0.1N trichloroacetic acid[6]. Afterwards the glucose concentration was measured by the GOD-Perid method[7] in each case by measuring the absorbance of Quinoneimine dye solution at 505 nm in a UV-Visible Spectrophotometer (Thermo, Genesis).

3. RESULTS AND DISCUSSION

The casein whey was straw coloured and was found to have a pH of 4.8. The lactose content was estimated using GOD-Perid method and has a concentration of 3.2% .

Michaelis-Menten equation was used to model the enzymatic hydrolysis of lactose solution

$$v = \frac{dP}{dt} = \frac{V_{\max} S}{K_m + S} \qquad (1)$$

where v is reaction rate, $\frac{dP}{dt}$ is rate of product formation, V_{max} represents maximum rate achieved by the system at maximum (saturating) substrate condition, Michaelis constant K_m is the substrate concentration at which the reaction rate is half of V_{max}. K_m is a reflection of the affinity of enzyme for its substrate and is characteristic for a particular enzyme-substrate system. The smaller the value of K_m, the more strongly the enzyme binds the substrate[8].

The parameters $V_{\max}$ and K_m were estimated using Lineweaver-Burk plot[9]. From Fig.4 the values of $V_{\max}$ and K_m have been estimated and those are found to be 4.38 mmol/l/min and 64.034 mM respectively. The small value of K_m signifies that enzyme-substrate binding is sufficiently strong.

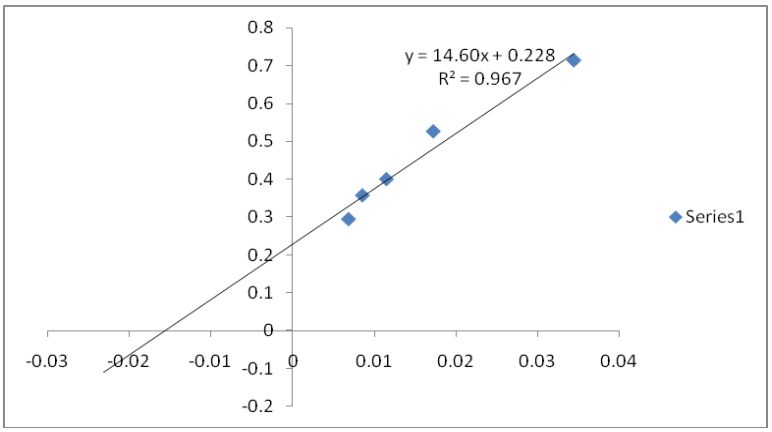

Fig. 4: Line-weaver –Burk plot at 32^0C

The catalytic efficiency of the reaction was calculated by the following Equation:

$$k_{cat} = \frac{V_{\max}}{[E_0]} \quad (2)$$

where $V_{\max}$ is maximum reaction velocity, $[E_0]$is total enzyme concentration and k_{cat} is catalytic efficiency. The catalytic efficiency was found to be 38.81 min^{-1}.

4. CONCLUSION

In this study, the lactose content of casein whey, a dairy effluent has been estimated to be 3.2%. For proper utilization of whey, enzymatic hydrolysis was carried out. Product glucose concentration was found. The kinetic study of enzymatic hydrolysis reaction reveals that Michaelis Menten equation has been followed. The parameters of $V_{\max}$ and K_m have been found to be 4.38 mmol/l/min and 64.034 mM respectively. Catalytic efficiency was also reported.

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