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Original Paper

Production of simple sugars from lactose and lactoserum

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Abstract
In this work, we were interested in the valorization of dairy industry waste. The City of Rabat/Salè's Milk Processing Unit discharges approximately 7500 litres/day of whey daily. Due to its biochemical composition (lactose, proteins, vitamins), whey is an excellent recyclable medium and becomes a formidable pollution factor. Similarly, the production of bioethanol through the fermentation of dairy industry waste is very limited. The yield and alcohol tolerance of the organisms in the alcoholic fermentation of lactose from whey is very low. So the objective of this work is to produce fermentable monomeric sugars fermentable in ethanol from whey. This study proposes chemical treatments of whey to release glucose and galactose. Also, the experimental conditions were optimized and the results of chemical hydrolysis by carbon dioxide, sulphuric acid and hydrochloric acid were compared. This study made it possible to develop processes for valuing dairy industry waste using clean technologies by reducing factors as pollutants and for the production of fermentable molecules.

1. Introduction

More than 80% of the world's energy resources are mainly of petrochemical origin. International recommendations commit all countries to reduce greenhouse gas emissions. This forces them to gradually move towards local and CO2-neutral energy sources [28,20,1]. Bioconversion of renewable feedstocks from biomass, such as agricultural and wood waste, into ethanol or other fuels can have significant environmental and economic benefits [14, 9,11].

The dairy industries produce hundreds of millions of kilograms of milk and its derivatives annually. This transformation results in the release of huge quantities of residues that present a considerable environmental problem [10,13,1]. The main discharges are white water, whey and buttermilk. The high BOD load of these discharges, greater than 50 g/L, is a serious problem since its direct discharge to sewage treatment plants is not only expensive but overloads the treatment facilities.

However, the lactose contained in this medium represents 90% of the BOD load, constitutes an excellent recoverable substrate and becomes a formidable pollution factor [16, 21, 3,11].

Due to the properties of yeasts, whey can be fermented directly into ethanol, but the yield is lower than traditional fermentation from glucose [6,22,1]. To this end, in this work, the waste from milk processing has been chemically treated and hydrolyzed for the production of monomeric sugars. These sugars were then used as a carbon source for the production of bioethanol by fermentation. The optimization of the experimental conditions was studied and the results of the chemical hydrolysis were compared.

2. Experimantal details

2.1- Substrates

The substrate is obtained from rejects from the city's large dairy industry Rabat/Salé. This substrate has been stored at 4°C until use. The experiences were also performed on pure lactose solutions. Several concentrations of lactose were obtained by mixing D-Lactose (Sigma Aldrich Ltd) with distilled water.

2.2- Hydrolysis by sulphuric acid, hydrochloric acid and carbon dioxide

The substrate was treated with sulphuric acid, hydrochloric acid and carbon dioxide using the techniques described by [6,19]. Acid concentration, temperature and processing time were optimized by the experimental planning method using the Carlson R. and Nordahl A. process, (1993).

2.3- Determination of effluent parameters

In order to determine the impact of L.V.R.S. and confirm the feasibility and results of the treatments performed, the composition of the whey, pH, COD, BOD5 and COD/BOD5 before and after the treatments were investigated using the techniques described by [6,1]

2.4. Determination of lactose and simple sugars

Sugars in different media are determined by the dinitrosalicylic acid (DNS) method described by [16]. It is based on the formation of a chromatophore between the reducing ends of sugars and dinitro-salicylic acid (DNS). In a 20 ml test tube containing 3 ml of Miller's reagent, 2 ml of the solution to be measured is added. After heating for 15 minutes at 100°C and cooling to room temperature, the absorbance is measured at 640 nm. A standard curve is prepared from a stock solution of 1g/l glucose with values from 0 to 1g/l. The phenol-sulphuric acid method is used to evaluate the spectrum of monomeric sugars derived from hydrolysate [8].

3. Results and discussion

This work is divided into two phases presented in Figure 1. The first part is concerned with hydrolyzing the filtered discharges from the L.V.R.S. processing industry to release simple sugars. The second part will study the fermentation of hydrolysate for the production of bioethanol. This second part is still being drafted.

3.1. Characteristics of L.V.R.S. processing whey

Several indicators will be used to assess whether an effluent can be considered as a pollutant. Tables I and II summarize some of the parameters of the L.V.R.S. effluent.

In whey, lactose represents 90% of the BOD load, which at the same time constitutes a valuable substrate and a real threat to the environment.

Consequently, these cheese factory effluents must undergo specific treatment in order to considerably reduce the organic load before being discharged.

Table I: Physico-chemical discharge characteristics of the L.V.R.S. processing industry using the techniques described by[6,1]

Type of effluent	pH	Volume produced per litre of milk	COD (gl)	COD/BOD5
White water	5.5 to 6.2	3 to 4	2 to 3	1.3 to 1.4
Whey	4,3	0,75	50 à 70	1.5
White water + whey	4 to 4,5	4 to 5	10 to 12	1,7 to 1,8

Table II: Reject whey composition from the L.V.R.S. processing industry

Constituents	Whey (g/kg)	References of the assay and analysis method
Dry matter	62	Schuck P. et al., 2004
Lactose	400	Dubois 1956; Butylina S. 2007; Miller GL. 1969
Proteins	82.5	Lajoie D., 1997 Bertrand M., 2002. Butylina S. 2007.
Minerals - ashes	41	Schuck P. et al., 2004
Organic acids	11	Wee et al., 2005; 2006
Fat content	2,1	Wee et al., 2005; 2006

Among the practices, filtration has made it possible to separate the components present in the environment. The membranes used have pores of different sizes depending on the substrate required. By this technique, L.V.R.S. discharges are essentially composed of water, lactose, proteins, minerals and fat. The results and references of the assay and analysis methods are presented in Table II.

3.2- Hydrolysis of lactose

Lactose is a diholoside (or disaccharide), composed of a molecule of β-D-galactose (Gal) and a molecule of α/β-D-glucose (Glc) linked together by an osidic bond β(1-4). The official name of lactose is β-D-galactopyrannosyl(1-4)D-glucopyranose. It can be symbolized by Gal β(1-4) Glc.

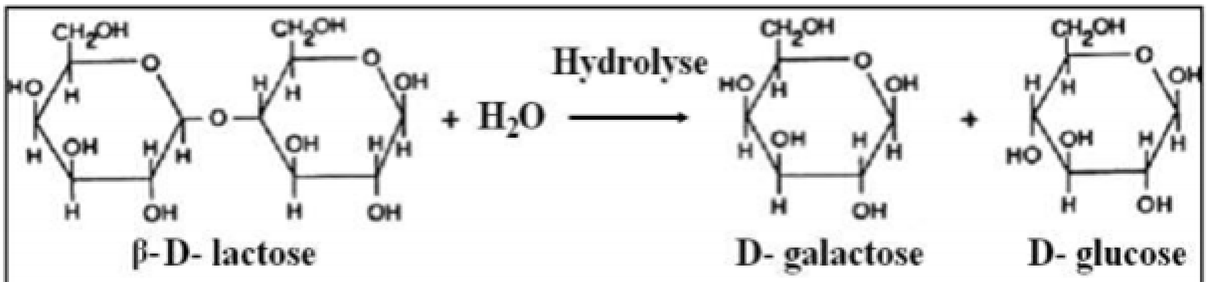

Figure 2: Hydrolysis of lactose in the presence of a water molecule.

The lactose contained in whey can be a good energy substrate [7, 18, 6]. Lactose processing is of great interest to the food industry. Lactose must first be segmented in the presence of a water molecule into D-glucose and D-galactose (Figure 2). Hydrolysis can be chemical by acids or biological by enzyme [23,27].

3.2.Hydrolysis by carbon dioxide (CO2)

In the first experiments we studied the feasibility of hydrolyzing whey with CO2 at different pressures (2758, 4137, and 5516 kPa(g)) and between 180°C and 220°C temperatures (Figures: 3; 4; 5). For the control, the experiments were performed in the absence of CO2 (Figure 4).

In these figures, we noted a change in the concentration of sugars (lactose, galactose and glucose) after the acid hydrolysis of the whey filtered by dioxide of carbon (CO2). Maximum concentrations are obtained after about 15 minutes of the experiment (Figures 3 and 4). Hence lactose is converted into two monosaccharides, galactose and glucose. Although in Figure 3 the concentration of galactose is 4 times higher than that of glucose (0.15 against 0.04 M) at 15 min.

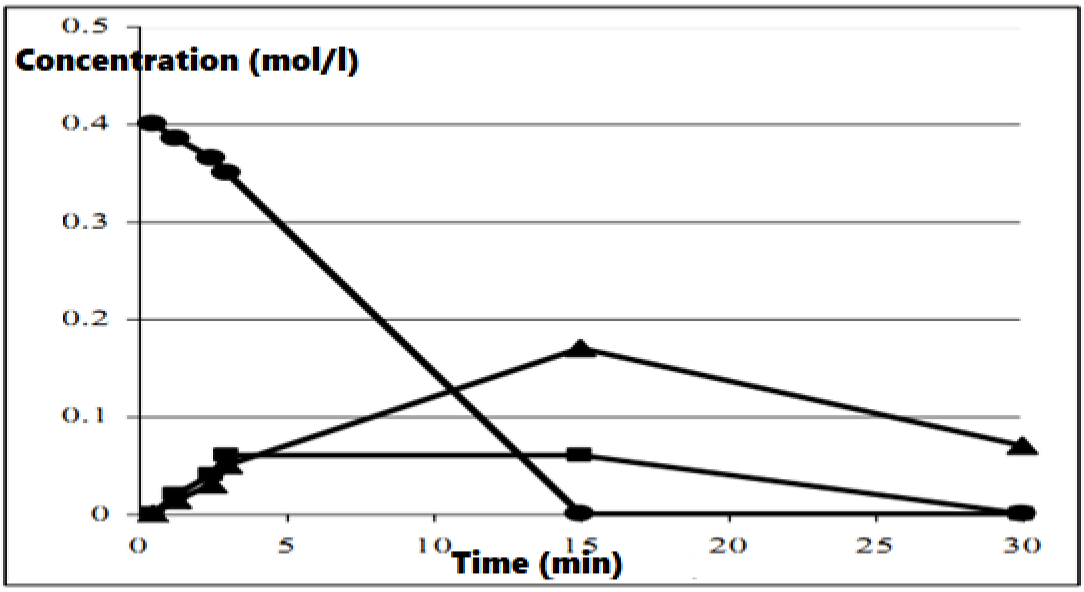

Figure 3: Hydrolysis of whey at 200°C and 2758 kPa(g) by carbon dioxide (CO2), lactose (●), galactose (▲) and glucose (■).

La production du galactose est augmentée en diminuant la température, en atteignant une conversion maximale de 47% à 180°C (Figure 5). Les deux sucres sont complètement décomposés au-delà de 15 min. Pour le contrôle, la figure 4 représente 'hydrolyse du lactose constitué par CO2.

C'est aussi, la formation des deux hexoses a été observée. En principe, le dioxyde du carbone a crée des conditions acidulants pour hydrolyser le lactose. Le CO2 dissous ne baisse pas le pH aux niveaux convenable pour Dissolved CO2 does not lower the pH to levels suitable for hydrolyzing lactose. Therefore, an increase in temperature was necessary for such hydrolysis. However, at these high temperatures the hydrolysis of lactose has been observed even in the absence of CO2. At high temperature the hydrolysis of the lactose does not require CO2 treatment (Figure 4), it is called a heat treatment.

Also at the natural pH of the lactose solution which is approximately 3.5 was suitable for catalyzing hydrolysis at 200°C. However, the addition of CO2 has decreased by in addition to the pH of the solution, thus increasing the hydrolysis rate.

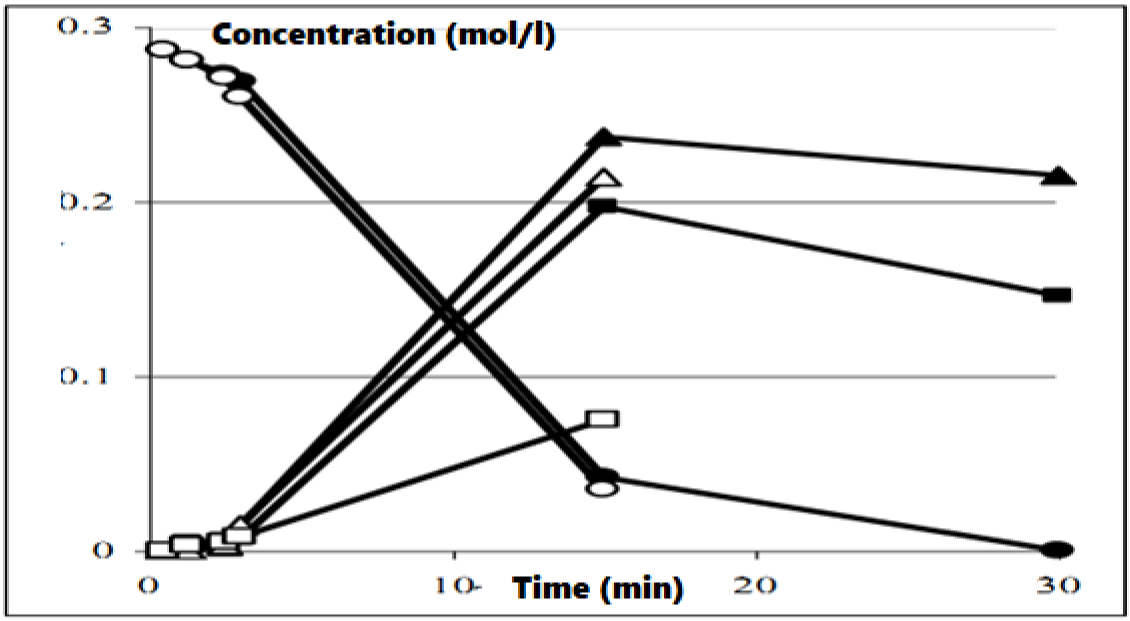

Figure 4: Hydrolysis of lactose consisting at 200°C and 5516 kPa(g) of carbon dioxide CO2 (dark symbol) and absence of CO2 (light symbol), lactose (●,○), galactose (▲,∇) and glucose (■,□).

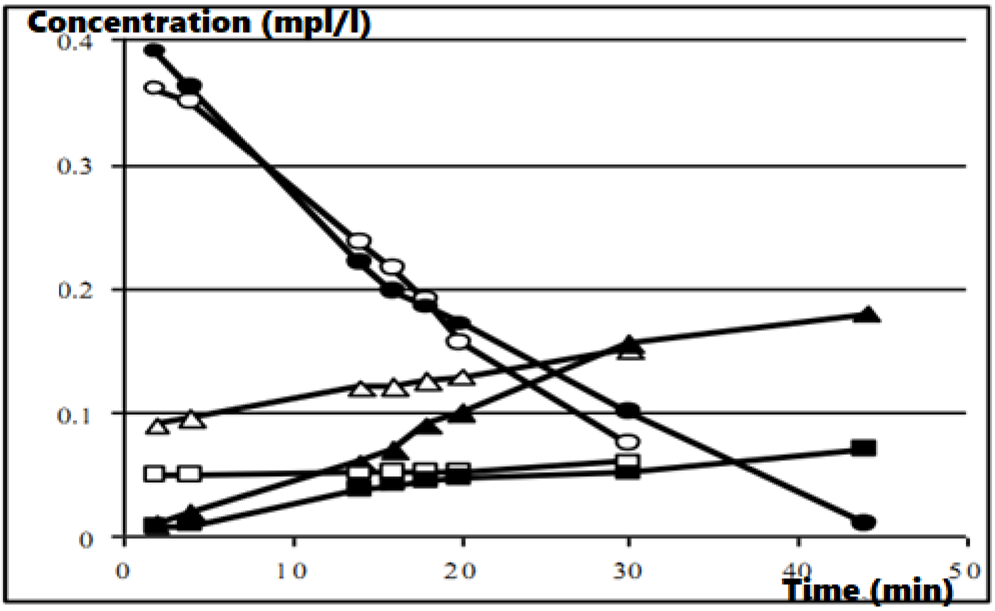

Figure 5: Comparison of lactose concentration (●,○), galactose (▲,▽) and glucose (■,□) at 180°C, during acid hydrolysis by CO at 4137 kPa(g) (Dark symbol) and by 0.2 M H SO

Similarly, lactose hydrolysis was observed without the addition of an acidulant (Figure 4). During this time, the hydrolysis rate was faster compared to that of whey. This result was unexpected because the natural pH of whey (~5.0) is higher than the natural pH of pure lactose solutions (~3.5). The only explanation is related to the effect and activity of the whey components mainly the minerals at the time of hydrolysis. Such remarks were mentioned in the literature from [12,2, 6].

3.4.Hydrolysis by sulphuric acid and hydrochloric acid

We compared the carbon dioxide effect with the effect of sulphuric acid (between 0.15 and 0.5M) and hydrochloric acid (between 0.5 and 1M) on the hydrolysis of whey. Under the same conditions, and to provide a pH of 3.7, we added 0.2M of H2SO4 and 0.9M of HCl. The results of this experiment are comparable to those produced by carbon dioxide (Figures 5 and 6). These figures represent typical curves obtained during hydrolysis. Lactose concentration decreases, while galactose and glucose concentrations increase.

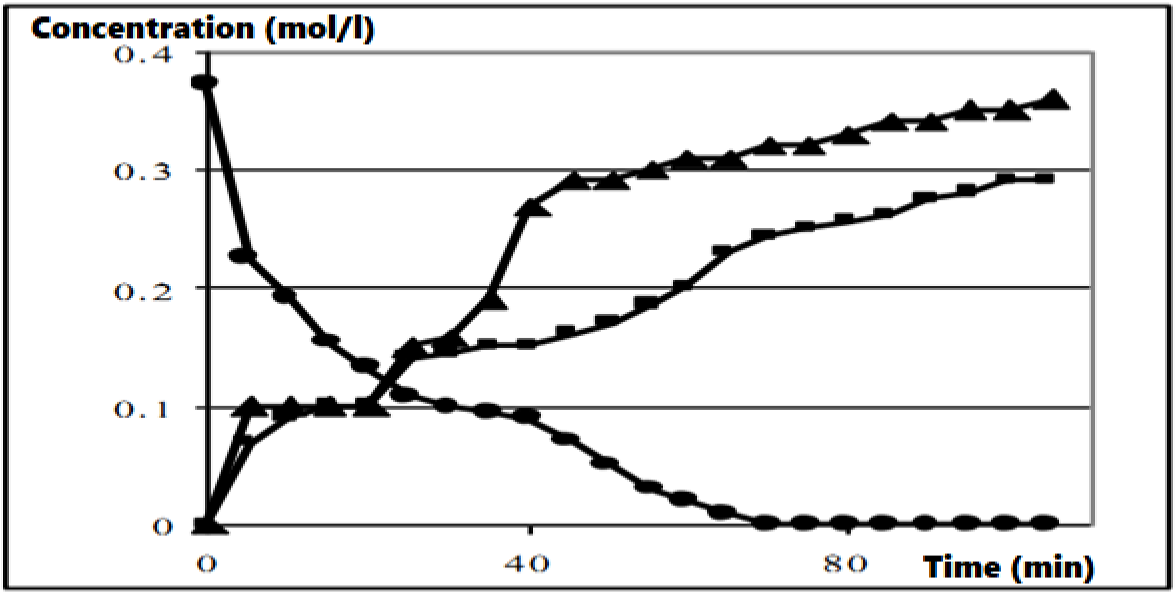

Figure 6: Concentration of lactose (●), galactose (▲) and glucose (■) during acid hydrolysis by HCl (1M) at 90°C.

Conclusion

The process of using whey as a source of substrate has the advantage of being depolluting and enhancing through the production of bioenergetic molecules fermentable in bioethanol. It is considered to be a very good form of renewable, clean energy that does not generate additional carbon dioxide (CO2) in the atmosphere. This type of process can also improve the income of industrial units in agricultural countries.

At the laboratory level, additional studies on other possibilities for improving yields and on new processes for recovering organic waste produced by the agri-food industries are being carried out, such as the hydrolysis of lactose by free and immobilized enzymes, the production of exopolysaccharides, bioethanol and biomethane.

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