DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ETHANOL PRODUCTION FROM CHEESE WHEY POWDER SOLUTION BY FERMENTATION

by
Serpil ÖZMIHÇI

March, 2009
İZMİR

ETHANOL PRODUCTION FROM CHEESE WHEY POWDER SOLUTION BY FERMENTATION

A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
In Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy in Environmental Engineering,
Environmental Sciences Program

by
Serpil ÖZMIHÇI

March, 2009
İZMİR

Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"ETHANOL PRODUCTION FROM CHEESE WHEY POWDER SOLUTION BY FERMENTATION"** completed by **SERPİL ÖZMIHÇI** under supervision of **PROF. DR. FİKRET KARGI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

Prof. Dr. Fikret KARGI
Supervisor

Prof. Dr. Sol Kohen ÇELEBİ
Committee Member

Prof. Dr. Rengin ELTEM
Committee Member

Prof.Dr. Tülin KUTSAL
Jury member

Prof.Dr. Adem ÖZER
Jury member

Prof. Dr. Cahit HELVACI
Director
Graduate School of Natural and Applied Sciences

ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof.Dr. Fikret KARGI for his guidance, motivation, valuable advises, encouragement and for his patience during the thesis.

I wish to thank the members of my thesis committee, Assoc. Prof. Dr. İlgi K. KAPDAN and Prof. Dr. Rengin ELTEM, for their contribution, guidance and support.

This thesis was supported in part by research funds of Turkish Prime Ministry State Planing Organization (Utilization of food industry wastewaters: Ethanol production from cheese whey." Project No: 2005K120360) and Dokuz Eyül University-Scientific Research Foundation (Comercial chemical (ethanol) production from food industry waste" Project No: 03.KB.FEN.001).

I would like to thank all my friends, especially to Dr. Serkan EKER, Dr. Yunus PAMUKOĞLU, and Ass. Prof. Görkem AKINCI, Dr. Duyuşen GÜVEN for their patience, moral support during the course of this study.

Special thanks to my family and my only nephew Başar ÖMÜRLÜ, waiting for me with a big patience to play with him, for their love and invaluable support.

I dedicate this thesis to my family.

Serpil ÖZMIHÇI

ETHANOL PRODUCTION FROM CHEESE WHEY POWDER SOLUTION BY FERMENTATION

ABSTRACT

Ethanol production from cheese whey powder (CWP) solution was investigated using batch, fed-batch and continuous fermentation systems. In batch experiments ethanol production from cheese whey, CWP and lactose solutions with the same initial sugar contents were compared by using two different Kluyveromyces marxianus strains (NRRL–1109, NRRL–1195) in order to determine the most suitable substrate and the yeast strain.

Then, the effects of initial pH, CWP concentration and external nutrient supplementation on ethanol production were investigated using K. marxianus NRRL-1195. The rate and extent of ethanol formation did not increase with external nutrient addition indicating no requirement for external nutrients. Final ethanol and the rate of ethanol formation increased with increasing CWP indicating no substrate or product inhibitions, but substrate limitations.

Performances of two different K. marxianus strains (NRRL-1195 and DSMZ-7239) were compared for ethanol fermentation. DSMZ-7239 was found to be the most suitable strain and was used in further experiments.

Effects of initial CWP and yeast concentrations were investigated and a kinetic model describing the rate of sugar utilization as function of the initial substrate and the biomass concentrations was developed in batch fermentation.

Then, a five- cycle repeated fed- batch operation with different feed CWP concentrations was used for the same purpose. The growth yield coefficient decreased and product yield coefficient increased with increasing feed sugar content.

A continuous culture at different feed sugar contents and hydraulic residence times (HRT) was tested for ethanol production. Material balances for yeast growth,

sugar utilization and ethanol formation with suitable kinetic models were used to predict the system performance and to determine the kinetic constants.

Finally, a continuously operated packed column bio-reactor (PCBR) using olive pits as support particles was used at different HRTs and feed sugar cotent. Sugar concentration decreased and ethanol increased with the height of the column operated in up-flow mode. Effluent ethanol increased with increasing HRT and feed sugar content up to certain levels. Ethanol yields closer to the theoretical predictions were obtained

Keywords*:* Cheese whey powder (CWP), ethanol fermentation, Kluyveromyces marxianus; batch fermentation, repeated fed-batch operation, continuous ethanol fermentation, packed-column bioreactor (PCBR), hydraulic residence time, feed sugar content, kinetic models.

PEYNİR ALTI TOZU ÇÖZELTİSİNDEN FERMENTASYONLA ETANOL ÜRETİMİ

ÖZ

Peynir altı tozu (PAT) çözeltisinden etanol üretimi kesikli, ardışık-kesikli ve sürekli sistemlerde incelenerek işletme parametrelerinin etkileri degerlendirildi. Öncellikle, kesikli deneylerde aynı şeker miktarını içeren peynir altı suyu, PAT ve laktoz çözeltileri iki farklı Kluyveromyces marxianus türü (NRRL–1109, NRRL–1195) kullanılarak karşılaştırıldı ve PAT'ın etanol üretimine uygunluğu tespit edildi.

Sonra, K. marxianus NRRL-1195 mayası kullanılarak giriş pH'ı, PAT derişimi etkileri ve ek nütrient gereksinimleri araştırıldı. Ek nütrient ile etanol hızının ve miktarının artmadığı görüldü ve böyle bir gereksinimin olmadığı sonucu elde edildi. Artan PAT miktarlarıyla oluşan etanol miktarının ve hızının arttığı, substrat ve ürün inhibisyonu olmadıgı sonucuna varıldı.

İki farklı K. marxianus türü (NRRL-1195, DSMZ-7239), PAT çözeltisinden etanol oluşum performansları açısından karşılaştırıldı ve DSMZ-7239 en uygun tür olarak saptanarak diğer deneylerde bu maya kültürü kullanıldı.

Kesikli fermentasyonda başlangıç PAT ve maya derişimlerinin etanol oluşumu üzerine etkileri araştırıldı. Etanol oluşum ve şeker giderim hızları, giriş substrat ve biyokütle derişiminin bir fonsiyonu olarak kinetik bir modelle açıklandı.

Kesikli deneylerden sonra, aynı amaçla beş-döngülü ardışık kesikli beslemeli işletilen bir fermentör kullanıldı. Artan giriş şeker derişimleriyle hücre büyüme katsayısı düştü ve ürün oluşum katsayısı arttı.

Sürekli kültürle alıkonma süresinin ve giriş şeker derişimlerinin sistem performansı üzerine etkileri etanol oluşumu için araştırıldı. Mayanın büyümesi, şeker giderimi ve etanol oluşumunu karakterize eden kinetik modeller geliştirildi ve model katsayıları saptandı.

Son olarak, zeytin çekirdeklerinin destek parçacıkları olarak kullanıldığı sürekli işletilen dolgulu bir biyo-reaktörde etanol fermentasyonu değişik alıkonma sürelerinde ve giriş şeker derişimlerinde incelendi. Yukarı akışlı çalıştırılan kolonda artan yükseklikle şeker derişimi azaldı ve etanol derişimi arttı. Çıkış etanol derişimi artan alıkonma süresi ve giriş şeker derişimiyle bir noktaya kadar arttı. Teorik verime yakın etanol oluşum verimleri elde edildi

Anahtar sözcükler: Peynir altı tozu (PAT), etanol fermentasyonu, Kluyveromyces marxianus; kesikli fermentasyon, ardışık-kesikli işletme, sürekli etanol fermentasyonu, dolgulu kolon biyoreaktörü, hidrolik alıkonma süresi, giriş şeker derişimi, kinetik model

CONTENTS

Page

CHAPTER ONE
INTRODUCTION

1.1 The Problem Statement

Wastewater of food industry usually contains high concentrations of carbonaceous organic chemicals in form of carbohydrates and no toxic compounds which make them emendable for biological conversions. Wastewaters of dairy industry (milk-cheese-yoghurt), meat-poultry, starch, and fruit juice-soft drinks industry contain significant amounts of carbohydrates, proteins, fats-lipids that can easily be metabolized by special organisms and converted to useful products under special conditions. By using proper organisms and conditions it is possible to produce some commercial products such as ethanol, organic acids (lactic, acetic etc), and high protein animal feedstuff (single cell protein) from these wastewaters some of which may require pre- treatment before bio-conversion. (Mielenz, 2001; Hari et al., 2001; Nigam, 2000; Gong et al., 1999; Cheung and Anderson, 1997; Agu et al., 1997; Lark et al., 1997; Duff and Murray, 1996; Zayed and Meyer, 1996; Palmqvist et al., 1996)

Ethanol is one of the most important chemicals that can be produced from carbohydrate rich wastes. The reason for the current interest on ethanol production, which is the main goal of this study lies on the extensive use of ethanol. Biofuels can replace petroleum in today's vehicles as a main transportation fuel. Automakers are encouraged to produce flex-fuel cars, which can use 100% ethanol instead of gasoline.

Ethanol is mainly produced from agricultural sources in the world. Production of ethanol from starch containing materials is technically feasible. However, high water requirement in irrigation (to grow the corn necessary to produce one gasoline gallon-equivalent of ethanol requires about 2,700 gallons of water), high cost of corn and other starch containing grains makes the process economically less attractive. Also, not having sufficient farm land is the main problem for ethanol production as discussed in the world especially after the food crisis in 2007. It has been estimated that converting the entire U.S. corn crop to ethanol would only yield energy equal to

12 percent of gasoline consumption and would fall far short of the 2017 goal. (Natural Gas vehicles for America, 2008)

Utilization of waste materials for ethanol production eliminates all the irrigation problems and offer special advantages by providing cheap raw materials and simultaneous waste treatment with ethanol production. Waste biomass has been the most widely used raw material for production of ethanol. However, ethanol production from waste biomass is expensive since the process requires separation of lignin from cellulose, hydrolysis of cellulose to sugars, fermentation of sugar solution to ethanol and separation of ethanol from water. Among the inexpensive and highly available raw materials for ethanol production are molasses and cheese whey, which are the waste by-products of sugar and dairy industries.

Cheese whey (CW) is a by-product generated in cheese industry. Production of cheese whey in the world is estimated to be over 10^8 tons per year. Because of its high organic content, whey imposes an important load on sewage treatment plants, and gives a big load to the environment, a common practice in underdeveloped areas, causes serious environmental problems. In addition to its main carbohydrate, lactose, cheese whey also contains proteins and vitamins. Cheese whey has been used by many investigators for production of ethanol because of its high carbohydrate content and availability. (Moulin et al., 1980; Maiorella and Castillo, 1984; Mahmoud and Kosikowski, 1982; Terrel et al., 1984; Chen and Zall, 1982; Marhawa and Kennedy, 1984; Marehawa et al., 1988; Cheryan and Mehaia, 1983). However, low concentration of lactose (5 to 6%) and therefore ethanol makes the recovery expensive. Ultrafiltration and drying techniques have been used to concentrate CW to be a raw material in ethanol production. (Domingues et. al., 2001; Kourkoutas et al., 2002; Silveira, et al., 2005; Grba et al., 2002; Zafar & Owais, 2006, Ling K.C.,2008).

1.2 Ethanol As A Chemical and Energy Source

Ethanol is widely used for sanitizing, cleaning and as a solvent. Also it's an additive of perfumes, paints, spirits, foodstuffs, antiseptics and fuels. Ethanol is also vital for the chemicals, pharmaceuticals, disinfectants, adhesives, cosmetics,

detergents, explosives, inks, hand cream, plastics and textile industries.(Addison K., 2008; Spectrum Chemicals & Laboratory Products, 2008)

Ethanol is a flammable, colorless liquid with a special odor. Ethanol contains a hydroxyl group, -OH, bonding to a carbon atom (CH_3CH_2OH). Its boiling and melting points are 78.5°C and -114.1°C respectively and has a density of 0.789 g ml^{-1} at 20°C (Spectrum Chemicals & Laboratory Products, 2008). Ethanol is a non-corrosive and relatively non-toxic alcohol made from renewable biological feedstock (bio-ethanol), by catalytic hydration of ethylene (ethylene $CH_2=CH_2$) with sulfuric acid from petroleum and other sources or by ethylene or acetylene from calcium carbide, coal or oil gas. (Kosaric, 2003; Wikipedia, 2008). Procedure of ethanol production includes microbial (yeast) fermentation of carbohydrates such as glucose distillation and denaturing. (Wikipedia, 2008)

Ethanol is used directly as fuel or as an octane-enhancing gasoline additive. Approximately 12 % of all U.S. gasoline contains ethanol at a blending percentage of 10%. Ethanol as a much cleaner fuel has major advantages over gasoline. Ethanol is a renewable and biodegradable energy source with less greenhouse effects as compared to gasoline. With an octane rating of 113, ethanol can be used as octane improver and ethanol blends can be used in automobile engines without much modification except at low temperature climates. Ethanol blends contain more oxygen resulting cleaner burning in engines and help to operate with optimal performance. Ethanol blends reduce hydrocarbon, nitrogen oxide (up to %20 with high level ethanol blends), carbon dioxide (100% on a full life cycle basis), volatile organic carbon compound (with high level ethanol blends 30%) emissions affecting on depletion of ozone layer. Sulphur dioxide, particulate matter (PM), cancer-causing benzene and butadiene (more than 50%) emissions are reduced by using ethanol blends (Addison K., 2008; Reed, 1981; Southridge Ethanol Inc., 2008; Mandil C., 2004; Hansen A.C. et.al., 2005).

1.3 Ethanol Fermentation Methods

Briefly, fermentation is the conversion of carbohydrates (sugar) into organic acids or alcohols under anaerobic conditions. Fermentation occurs under special conditions

requiring specific pH, oxidation-reduction potential (ORP), temperature, dissolved oxygen and nutrients, which need to be closely monitored. To obtain pure products, caution is needed to avoid contamination or to ensure that no anti-microbial reactions will occur. Toxic by-products and considerable waste may be produced at the end of fermentation. The fermentation reaction (glycolysis) including ethanol production is summarized in Figure 1.1. (Yim G & Glover C, 2008)

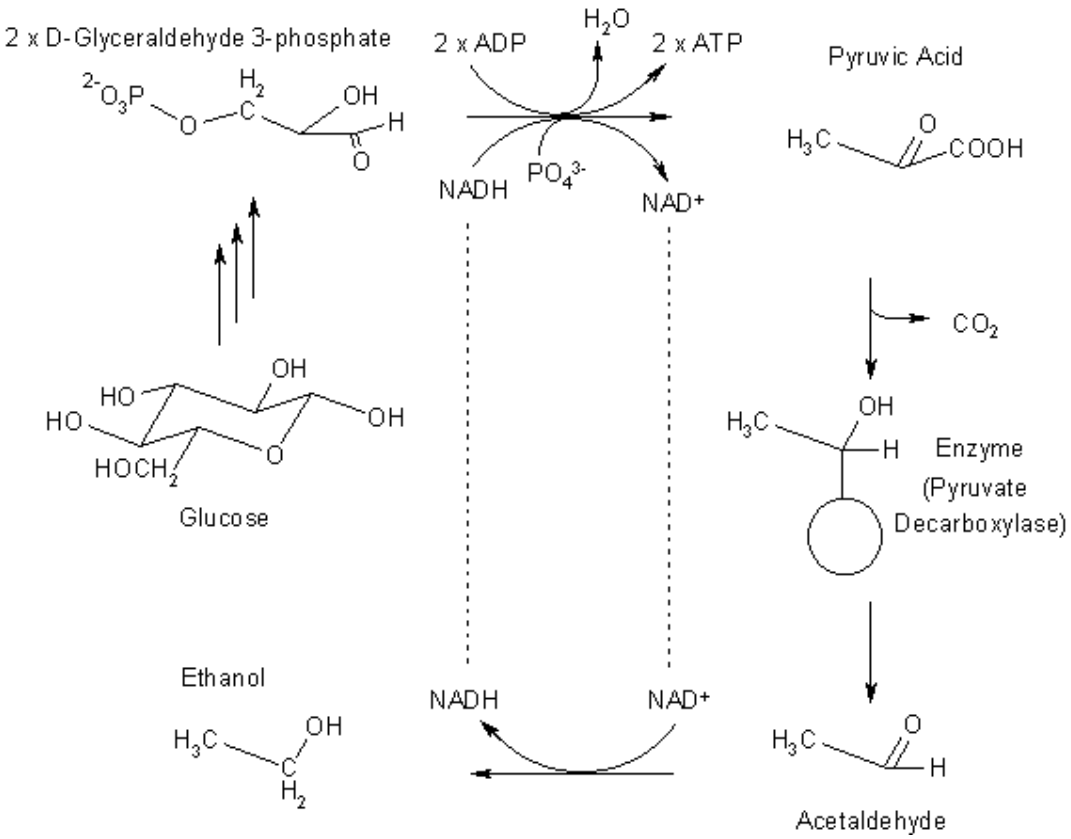


Figure 1.1 The fermentation of glucose to ethanol (Yim G & Glover C, 2008)

Ethanol fermenting organisms are mainly yeasts such as *Saccharomyces cerevisiae, S. uvarum, Schizosaccharomyces pombe*, and *Kluyueromyces sp.* Some bacteria can also ferment ethanol such as *Zymomonas mobilis, Clostridium sporogenes, Clostridium indolis* (pathogenic), *Clostridium sphenoides, Clostridium sordelli* (pathogenic), *Spirochaeta aurantia, Spirochaeta stenostrepta, Spirochaeta litoralis, Erwinia amylovora, Leuconostoc mesenteroides, Streptococcus lactis,* and

Sarcina ventriculi. Many of these microorganisms, generate multiple end products in addition to ethanol. (Najafpour G.D. et.al ,2002)

Cheese whey, which is used in this study, contains lactose that is a disaccharide and needs to be broken down into monosaccharides before fermentation. A lactose-fermenting organism has to include the enzyme beta-galactosidase to break down lactose into glucose and galactose. Glucose can enter glycolysis and the galactose can be converted into glucose.

Lactose fermenting organisms are *Saccharomyces cerevisiae, S. uvarum, Schizosaccharomyces pombe, Kluyveromyces sp. K. marxianus, K. kefyr and Torula cremoris. Kluyveromyces sp* are known to ferment lactose better than the other yeast strains for ethanol production.

1.3.1 Fermentation Mechanism of Kluyveromyces Spacie

Kluyveromyces includes two genes, LAC12 and LAC4 that hydrolyses lactose into glucose and galactose. Lac12p has an optimal pH for lactose uptake of 4.7 and the activity of hydrolising lactose can be saturated, requires energy, and probably uses H^+ or Na^+ ions. Figure 1.2 depicts a brief explanation of a theoretical model for the regulation of lactose permeabilization and hydrolysis in *Kluyveromyces*. Lac12p lets lactose and/or galactose enter the cells through basal levels of the lactose permease, then cytosolic Lac4 h-galactosidase hydrolyzes lactose into glucose and galactose. Glucose enters glycolysis directly, and galactose is converted into glycolytic intermediate, glucose- 6- phosphate through Leloir pathway. Galactose and ATP interacts with the bifunctional galactokinase, KlGal1p (the first enzyme acting in the Leloir pathway). KlGal1p leads to a conformational change that facilitates the interaction of the protein with the transcriptional repressor, KlGal80p. KlGal80p nuclear levels is reduced with cytosolic sequestration of KlGal80p into a complex with KlGal1p. Then the transcriptional activator specific of LAC/GAL gene, (KlGal4p) is released from the inhibition media by its interaction with KlGal80p. KlGal4p activates LAC gene expression through its binding as dimer to each of four specific upstream activating sequences (shown with dark gray bars), located in a common intergenic promoter region. In the other hand, glucose inhibits

the central regulator kinase KlSnf1p. KlSnf1p increases levels of active KlMig1p in the nucleus. KlMig1p, binds to an upstream repressor sequence in the KlGAL1 promoter, inhibiting its expression. This impairs KlGal1p-dependent release of KlGal4p from KlGal80p repression, finally resulting in the shutting-off of the GAL/LAC regulon. (Texeira M. R. ,2006; Domingues L., 1999, Ornelas A.P. ,2009)

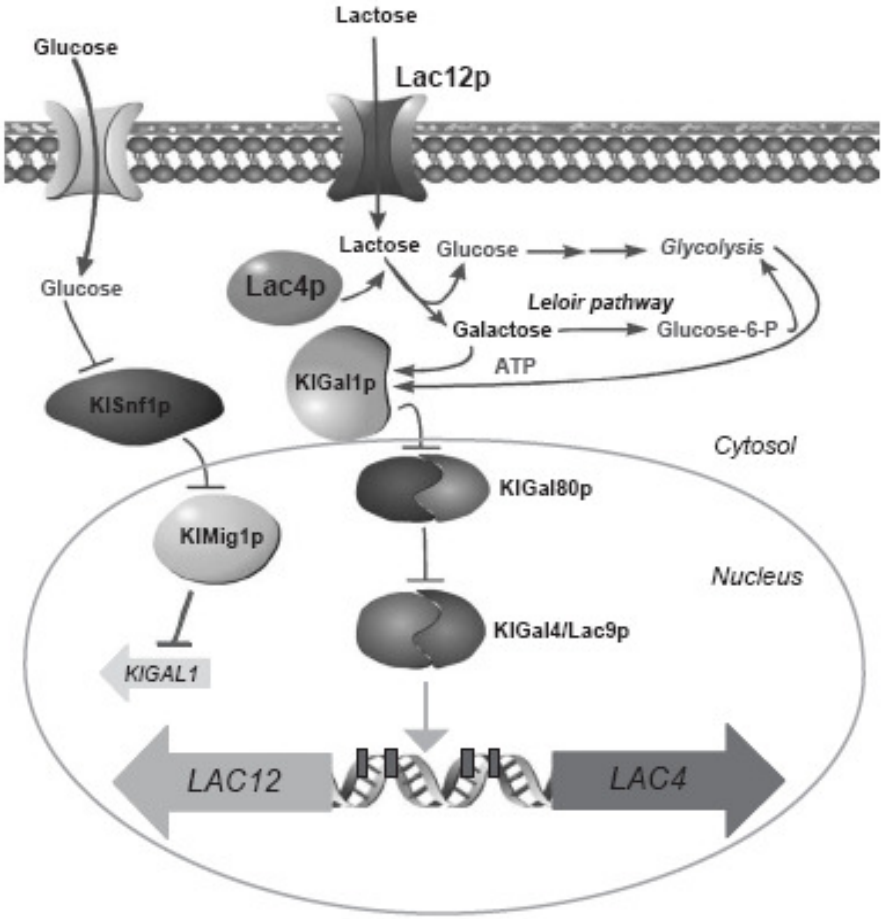


Figure 1.2 Model for the regulation of lactose permeabilization and hydrolysis in Kluyveromyces. (Texeira M. R., 2006)

1.4 Raw Materials For Ethanol Fermentations

Bio-ethanol is widely produced from a variety of feedstocks such as sugar cane, bagasse, miscanthus, sugar beet, sorghum, grain sorghum, switchgrass, barley, hemp, kenaf, potatoes, sweet potatoes, cassava, sunflower, fruit, molasses, corn, stover, grain, wheat, rice, straw, cotton, waste paper, cheese whey (contains about 6%

solids, of which three- fourth is lactose), other biomass, as well as many types of cellulose waste. The production of crystalline sucrose yields a by-product, molasses, which until recently has been the cheapest source of fermentable sugar. (Wikipedia, 2008, Reed, 1981; Mielenz 2001; Hari et al. 2001; Nigam 2000; Gong et al. 1999; Cheung and Anderson 1997; Agu et al. 1997; Lark et al. 1997; Duff and Murrey 1996; Zayed and Meyer 1996; Palmqvist et al. 1996; Siso 1996; Lightsey 1996, Sa´nchez O.J., Cardona C.A, 2008)

It is assumed that 45 kg of fermentable sugar such as glucose yields 18-23 kg of ethanol. Starch which has been gelatinized by heating can be readily hydrolyzed to fermentable sugars by enzymes. Starch is present in cereal grains like rice, wheat, corn, root crops, or potatoes. All of these are used in beverage fermentation. For starchy materials, the yield is between 40-50% based on the dry weight of carbohydrate. Complete hydrolysis of 45 kg of starch yields about 50 kg of glucose, but conversion is never complete, and with a 90% conversion the yields will be as indicated. For cellulosic materials, the yields of ethanol are substantially less because α-cellulose is quite resistant to enzymatic attack. Cellulosic materials containing α-cellulose, hemicellulose and lignin are present in saw mill residue, paper mill residue, newsprint, potato peelings, rice straw, corn stover, peanut shells, cocoa and coffee husks, tobacco stalks, wheat straw etc. (Reed, 1981; Sa´nchez O.J., Cardona C.A, 2008)

1.5 Cheese Whey and Cheese Whey Powder as Raw Material

Cheese whey is an important source of environmental pollution since 10 liters of cheese whey is produced from 1 kg cheese with high carbohydrate, protein and lipid contents. In the United States 16 million tons of cheese whey are produced from the annual production of about 1.6 million ton of cheese which could provide 378.5 million liters of ethanol annually. In Turkey, 700-800 thousand tons of cheese is produced per year forming approximately 7 million tons of cheese whey. (Reed, 1981, Tan S & Ertürk Y, 2002) It's estimated that a total of 51.6 billion liters of whey is generated in the world as a by product of cheese production in 2006, comprising about 48.9 billion liters of sweet whey and 2.8 billion liters of acid whey.

Due to high COD content of nearly 80 g l^{-1}, cheese whey is considered as a high strength wastewater from environmental point of view. Therefore, biological treatment of cheese whey by conventional activated sludge processes is very expensive (approx. 50 cents kg^{-1} COD). Anaerobic treatment of cheese whey is economically more attractive due to production of energy rich methane. Production of valuable chemicals from cheese whey has been considered as an attractive option because of its rich nutrient content. In addition to its main solute component lactose, proteins and vitamins are also present in cheese whey. However, low concentration of lactose and the produced ethanol makes ethanol recovery expensive. (Ozmıhçı S. & Kargı F., 2008)

Whey is mainly used as a food ingredient after drying. Highly-nutritious whey protein content and the presence of mineral salts and vitamins make whey particularly attractive for many branches of both the foodstuffs and the animal fodder industries. (Sienkiewics T., 1990) Concentrating, drying and fermentation of whey, delactosed, demineralized, deproteined or isolation of the individual whey constituents have been practiced largely. Whey is adaptable to ultrafiltration, reverse osmosis, ion exchange, electrodialysis and nanofiltration. Highly nutritious whey powder is widely used in the food industry.

Advantages of utilization of whey as a food material are summarized below, (Tadeusz S., Carl-Ludwig R., 1990; Ling K.C., 2008)

- Less pollution from cheese factory effluent
- Could be saled as typical whey products such as whey proteins, whey cream, lactose and milk minerals
- New whey products.

Whey can be classified as rennet whey (obtained during casein and cheese production) and acid whey. Also with factoring, technical whey can be also obtained from cheese whey.

Different procedures for the biotechnological utilization of whey to recover proteins, biomass, ethanol, organic acids have been proposed, but those processes require expensive operations of concentration, drying or fermentation. (Rubio-Texeira, 2000)

Whey resulting from the manufacture of cottage or cream cheese contains more lactic acid and correspondingly less lactose than the whey from certain Italian cheeses, cheddar cheese, or Swiss cheese. The protein content of whey produced in the manufacture of cream cheese, ricotta cheese or cottage cheese is lower. An inspection of the data on composition of whey indicates that lactose is the only fermentable carbohydrate in whey and composition of the whey vary depending on the source.

Composition of the two different cheese whey are given in Table 1.1 a and b.

Presence of only about 4.9% lactose also limits use of whey for fermentation purposes. Concentration of whey can serve to increase the content of lactose. Cheese whey is evaporated in ordinary conditions to produce cheese whey powder which is the condensed form of cheese whey. Cheese whey powder contains all the lactose content of cheese whey. (Tadeusz, Carl-Ludwig., 1990, Marth, 1973)

Concentrating by evaporation or reverse osmosis, drying, demineralizing by ion exchange or electrodialysis, ultrafiltration, air-drying, fermentation, crystallization, hydrolysis are the major processes used in utilization of cheese whey. (Tadeusz, Carl-Ludwig., 1990) Figure 1.3, summarizes cheese whey products used in foods. As seen from the figure, cheese whey can be used as animal feed without any processing. Cheese whey can be used in many different ways like as whey cheese, butter and drinks in food industry.

Figure 1.4 summerizes alcoholic, non alcoholic bevarages and drinks with whey additives that can be produced from whey. Also, whey powders and lactose are other alternative products obtained from cheese whey. Chemical and fuel industries use cheese whey and its products for alcohol, methane, organic acids, SCP, and whey syrups production (Tadeusz, Carl-Ludwig., 1990).

Table 1.1 Characterization of technical cheese whey
(a: Tadeusz S., Carl-Ludwig R.., 1990;
b: Ghaly, El-Taweel, 1997):

(a)

Charecteristics of whey		
Lactose (4-4,5%w/ v)	50000	mg l^{-1}
Protein (0.6-0.8% w/v)	9000	mg l^{-1}
mineral salts	(dry extract %8-10)	
BOD (30000-50000)	32000	mg l^{-1}
COD (60000-80000)	ca. 60000	mg l^{-1}
COD after milk protein removal	10000	mg l^{-1}
Phosphorus	150	mg l^{-1}
Nitrogen	1500	mg l^{-1}

(b)

Characteristics of whey		
pH	4.9	
Lactose	50	g l^{-1}
Total chemical oxygen demand	81050	mg l^{-1}
soluble COD	68050	mg l^{-1}
Insoluble COD	13000	mg l^{-1}
Percent soluble COD	85	
Total Solids	68300	mg l^{-1}
Fixed Solids	6750	mg l^{-1}
Volatile Solids	61550	mg l^{-1}
Percent volatile solids	90.1	
Suspended solids	25150	mg l^{-1}
Suspended fixed solids	220	mg l^{-1}
suspended volitile solids	24930	mg l^{-1}
percent suspended volatile solids	99.1	
Total Kjeldahl nitrogen	1560	mg l^{-1}
Ammonium N	260	mg l^{-1}
Organic N	1300	mg l^{-1}
Percent organic N	83.3	

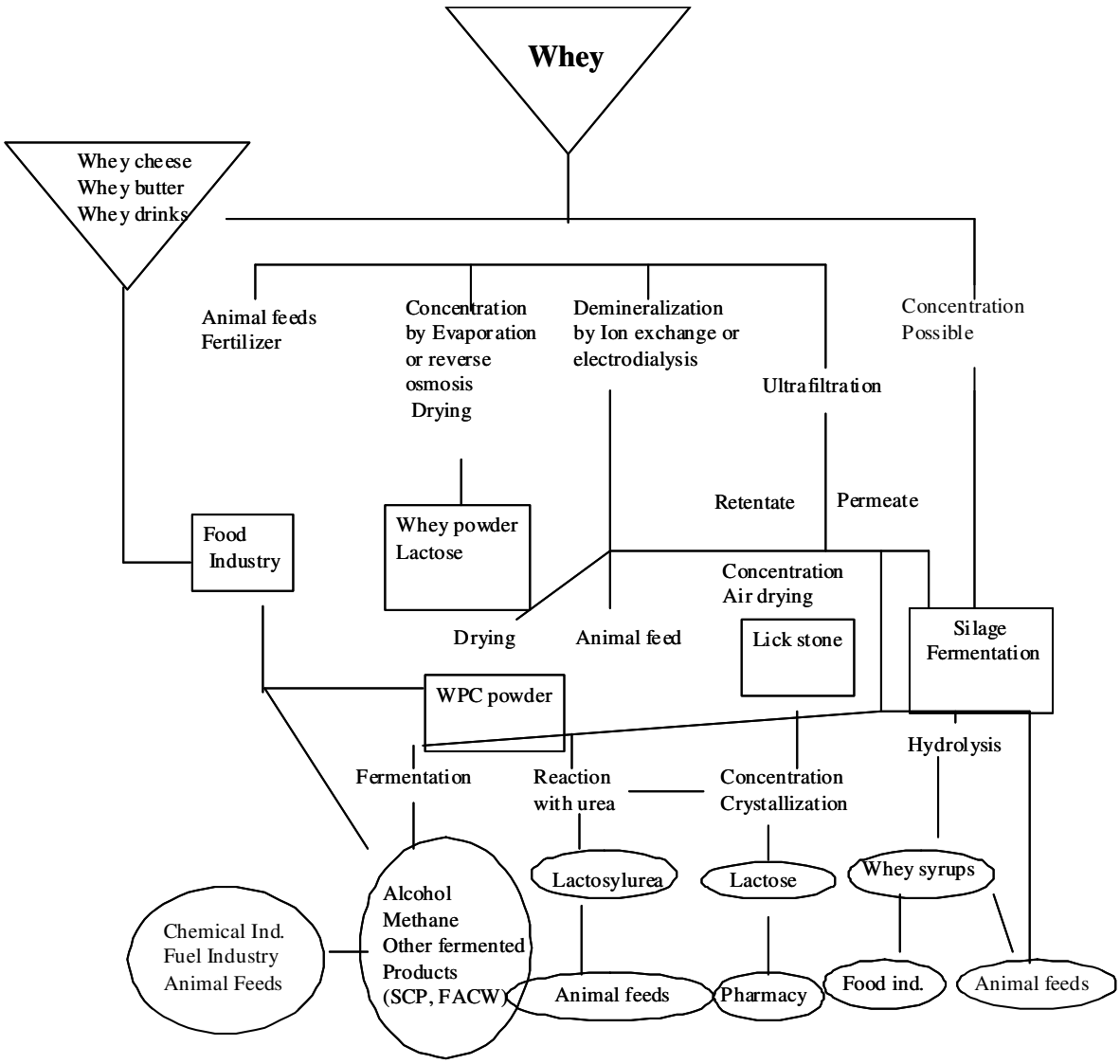


Figure 1.3 Whey processing for foods and feeds (Tadeusz, Carl-Ludwig., 1990)

Whey can also be used for production of yeast, ethanol, lactic acid and lactates, fermented whey beverages, non alcoholic beverages, alcoholic beverage, lactobionic acids, vitamin B_{12}, riboflovin, fat, penicilin, propionates, silage, vinegar, biogas (anaerobic operation) {Methane},2,3- butandiol, amino acids by fermentation (Tadeusz, Carl-Ludwig., 1990).

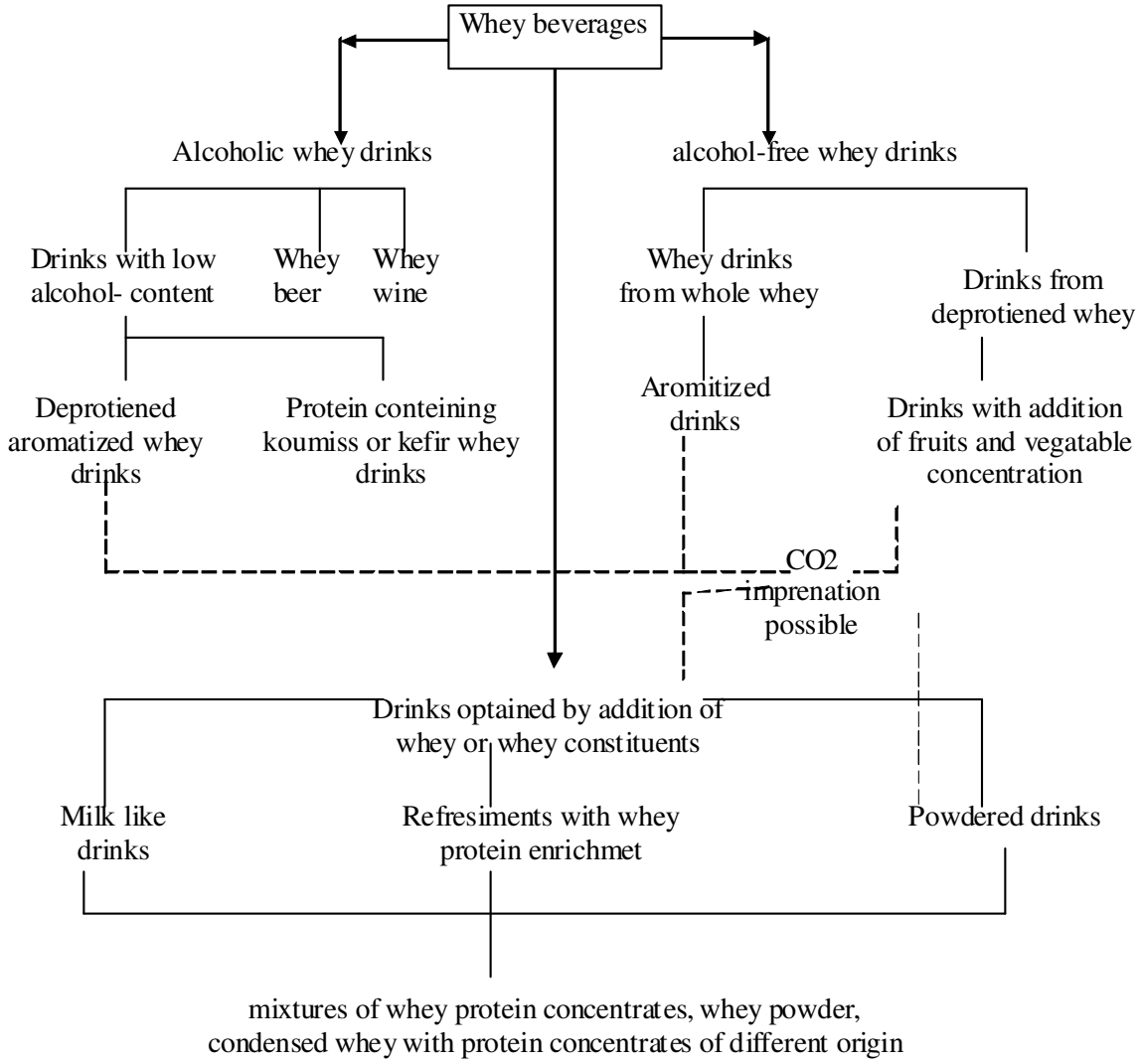


Figure 1.4 Classification of whey beverages (Tadeusz, Carl-Ludwig., 1990)

1.6 Ethanol Production Processs From Cheese Whey

Compared to fossil fuels ethanol has the advantages of produced from renewable sources, providing cleaner burning and producing low greenhouse gases. Ethanol, biogas, solvent feeds, polysaccarides, organic acids and their derivatives can be produced by utilization of lactose in whey. The theoretical yield obtained from 42 tonnes whey with 4.4 % lactose constitutes in 1 t. of 100 % alcohol since 0.54 kg alcohol can be theoretically produced from 1 kg lactose as presented by the following reaction (M. Altınbaş, 2002; Tadeusz, Carl-Ludwig., 1990)

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 4\ C_2H_5OH + 4\ CO_2$$

A great number of organisms are capable of ethanol formation. In addition to ethanol, other alcohols (butanol, isopropylalcohol, 2,3-butanediol), organic acids (acetic acid, formic acid, and lactic acids), polyols (arabitol, glycerol and xylitol), ketones (acetone) or various gases (methane, carbon dioxide, hydrogen) can be produced from CW by fermentation. The most known ethanol producing yeasts from lactose are *Saccharomyces cerevisiae, S. uvarum, Schizosaccharomyces pombe,* and *Kluyueromyces sp*. *K. marxianus*, *C. kefyr* and *Torula cremoris.* Mixed culture of *K. marxianus* and *Zymomonas mobilis* can also be used for ethanol fermentation. Yeast is a highly susceptible organism to ethanol inhibition, 1-2% (v v^{-1}) of ethanol retard microbial growth and 10% (v v^{-1}) alcohol stops the growth (Najafpour G. D. & Lim J.K., 2002; Tadeusz % Carl Ludwig, 1990; Hettenhaus J.R., 1998).

Ethanol production shown in Figure 1.5 includes the basic steps of the process. Whey is harvested from whey by ultrafiltration, then the remaining permeate is concentrated by reverse osmosis to attain higher lactose content. *Kluyveromyces* species added to fermentation media are pumped to the fermentation vessel. After fermentation, yeasts are separated and the remaining liquid is moved to the distillation process. Extracted ethanol is sent through the rectifier for dehydration. (Ling K. C., 2008; Tadeusz, Carl-Ludwig., 1990)

The first commercial operation from whey-to-ethanol (drinkable alcohol) plant is constructed in 1978 by Carbery Milk Products Ltd. in Ireland based on the main steps explained in Figure 1.5. After the the Carbery process developed in New Zealand and USA the company started fuel ethanol production in 1985. New Zealand started using fuel ethanol produced from whey in August 2007. (Ling K. C, 2008)

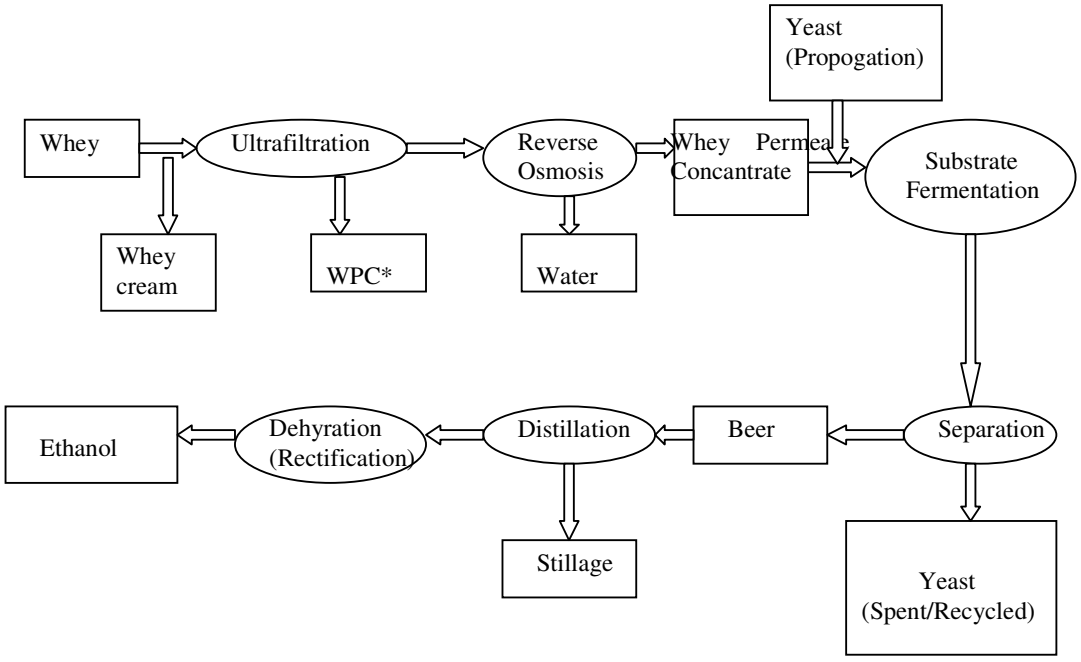


Figure 1.5 Basic steps of ethanol production from whey (Ling K. C., 2008; Tadeusz, Carl-Ludwig., 1990)

There are no reports in literature on utilization of cheese whey powder (CWP) solution for ethanol production other than our reported studies. (Kargi F. &. Ozmihci S ,2006; Ozmihci S. & Kargi F. ,2007a; Ozmihci S. & Kargi F. ,2007b; Ozmihci S. & Kargi F. ,2007c; Ozmihci S. & Kargi F. ,2007d; Ozmihci S. & Kargi F. ,2007e; Ozmihci S. & Kargi F. ,2008; Ozmihci S. & Kargi F. ,2009) CWP is a dried and concentrated form of cheese whey and contains lactose in addition to N, P and other essential nutrients. The use of CWP instead of cheese whey (CW) for ethanol fermentations has significant advantages such as:

- elimination of ultrafiltration processes used to concentrate lactose before fermentation
- compact volume
- long term stability
- high concentrations of lactose and other nutrients

Ethanol can be produced by applying mainly four types of operations in industry: batch, fed-batch, continuous and semi-continuous. Batch and continuous modes are most widely used processes. The Melle-Boinot process is one of the known batch ethanol fermentation process. Also, suspended and immobilized systems can be used. Cell recycle may advantageously be used with any of these operation modes. Simultaneous saccharification and fermentation can be used in cellulosic raw sources. All of the systems chosen have some advantages and disadvantageous depending on the raw material and species used. (Sa´nchez O.J., Cardona C.A, 2008)

Fed-batch operation for ethanol fermentations offer special advantages over batch and continuous operations by eliminating substrate inhibition as a result of slow feeding of highly concentrated substrate solution. Therefore, the growth and product formation rates can be controlled by controlling the substrate loading rate to the reactor. High cell density fed-batch reactors are used to improve productivity of conventional continuous fermenters. Most of the studies on cheese whey fermentations were realized by using batch or continuous fermentations. (Ozmihci S.&Kargi F., 2007c)

Continuous ethanol fermentations offer special advantages over batch and fed-batch operations by providing constant effluent quality, high productivity and control over the product concentration by adjusting the feed sugar concentration and the operating HRT. Continuous fermentations of ultrafiltered cheese whey were reported in literature with low ethanol yields. (Ozmihci S.&Kargi F., 2007d)

Biofilm cultures offer specific advantages over suspended cultures for ethanol fermentations from concentrated CWP solution such as providing high biomass concentration, high fermentation rate, compact reactor volume and reduced ethanol inhibition due to biofilm formation. (Ozmihci S.&Kargi F., 2008)

Different types of fermentors were used in ethanol production such as multistage perforated plate column fermentor, continuous stirred tank reactor with yeast recycle, whirlpool yeast separator, partial recycle reactor, APV tower fermentor, high cell density plug fermentor, continuous vacuum fermentation, continuous flash fermentation, continuous solvent extraction fermentation, membrane fermentor,

pressure membrane fermentor, rotor fermentor and hollow fiber fermentor. (Hettenhaus J.R., 1998)

1.7 Separation of Ethanol

Ethanol can be used alone as a fuel in form of a mixture of 95.6% w w^{-1} (96.5% v v^{-1}) ethanol and 4.4% w w^{-1} (3.5% v v^{-1}) water. However, in order to burn ethanol with with gasoline in automobile engines water needs to be separated. There are many dehydration processes to remove the water from ethanol/water mixture. These are fractional distillation, azeotropic distillation (adding benzene or cyclohexane to the mixture and forming heterogeneous azeotropic mixture in vapor-liquid-liquid equilibrium); extractive distillation (adding a ternary component increasing ethanol relative volatility. When the ternary mixture is distilled, it will produce anhydrous ethanol on the top stream of the column); molecular sieves (Ethanol vapor under pressure passes through a bed of molecular sieve beads. The bead pores are sized to allow absorption of water while excluding ethanol. After a period, the bed is regenerated under vacuum to remove the absorbed water); desiccation using glycerol; dehydration using adsorbents and vacuum separation. Molecular sieves compared to distillation methods can account 3,000 btus $gallon^{-1}$ for energy saving. (Wikipedia, 2008; Hansen A.C. et.al., 2005)

Adsorption techniques like activated carbon adsorption needs separation of ethanol from the adsorbent. Membrane separation is possible with pervaporation of water/ ethanol mixture. The media is heated in a reactor set near the fermentor and filtered through the membrane. The required characteristics of membranes are: high separation factor (a), high permeation rate (*P*), and high separation index (a*P*), as well as good mechanical strength and stability. Only membranes based on crosslinked poly -vinyl alcohol, chitosan, alginic acid, and poly -acrylic acid polyion complexes are acceptable for industrial application which requires over a 500 kg m^{-2} h^{-1} separation index for the dehydration of concentrated ethanol solutions. In addition, in some studies, the fermentor with thermophilic organisms was heated and separation occurred with vaporization. (Buyanov et.al.,2001; Iwatsubo et.al., 2002; Bruggen et.al., 2002; Gestel et.al., 2003; Geens et.al., 2004; Navajas et.al., 2002)

1.8 Energy and Economics of Ethanol

The economics of ethanol lies on “net energy” estimated with the energy inputs and outputs involving in ethanol production. The inputs are; the energy used to grow the raw material (if agricultural sources are used), to manufacture and to transfer the ethanol. Also the equation has to allocate the energy used in steps of ethanol production and the other by-products produced from the raw material. Some studies investigated with corn, showed that 1 BTU gal^{-1} ethanol is equal to 277.63 J l^{-1}. For most raw materials (for instance molasses or glucose syrups), it is essential that the plant be located close to the source of the raw material. The conduct of the fermentation is important for the overall cost. For dilute media, the rate of fermentation may be high, but fermentor productivity may be relatively low and the cost of distillation will be high because of the low concentration of ethanol. For media containing more than 10-15 % fermentable sugar, productivity in batch fermentation will also be low because of the inhibition effects of ethanol, but distillation cost will be lower. For continuous fermentation with cell recycle fermentation rates will be high and productivity will be excellent, but at higher dilution rates yield may be low. (Reed, 1981; Mandil C, 2004)

Biofuel production in the world is mainly based on agricultural sources. The energy balances of some developed countries; like the United States producing corn ethanol, Brazil producing sugarcane ethanol, Germany producing biodiesel are 1.3, 8, and 2.5 respectively. In literature also energy balance of cellulosic ethanol in USA was determined with experimental results depending on production method is in a range of 2 to 36. Ethanol production by the USA and Brazil are compared briefly in Table 1.2 where ethanol is produced from maize (USA) and sugar cane (Brazil) with a net energy balance of 1.3-1.6 times and 8.3- 10.2 times, respectively.

Table 1.2 Comparison of ethanol production in U.S.A. and Brazil (Renewable Fuels Association, 2008)

Comparison of key characteristics of the ethanol industries in the United States and Brazil			
Characteristic	**Brazil**	**U.S**	**Units/comments**
Feedstock	Sugar cane	Maize	Main ethanol production sources
Total ethanol production (2007)	5,019.20	6,498.60	Million U.S. liquid gallons
Total farm land	355	270[(1)]	Million hectares.
Total area used for ethanol crop (2006)	3.6 (1%)	10 (3.7%)	Million hectares (% total arable)
Productivity per hectare	6.8-8	3.8-4	tons of ethanol per hectare.
Energy balance (input energy productivity)	8.3 to 10.2 times	1.3 to 1.6 times	Energy produced / Energy expended
Flexible-fuel vehicle fleet (autos and light trucks)	6.2 million (E100)	7.3 million (E85)	
Ethanol fueling stations in the country	33,000 (100%)	1,700 (1%)	Brazil for 2006, U.S. as July 2008 and total of 170,000
Ethanol's share within the gasoline market	50% (April 2008) [(4)]	4% (December2006)	As % of total consumption on a volumetric basis.
Cost of production (USD/gallon)	0.83	1.14	2006/2007 for Brazil (22¢/liter), 2004 for U.S. (35¢/liter)
Government subsidy (in USD)	0 [(5)]	0.51/gallon (April 2008)	
Import tariffs (in USD)	0	0.54/gallon	As of April 2008
Estimated greenhouse gas emission reduction	86-90% [(2)]	10-30% [(2)]	% GHGs avoided by using ethanol instead of gasoline, using existing crop land.
Estimated payback time for greenhouse gas emission	17 years [(3)]	93 years [(3)]	Brazilian cerrado for sugar cane and US grass land for corn. Assuming land use change scenarios.

Notes: (1) Only contiguous U.S., excludes Alaska. (2) Assuming no land use change (3) Assuming direct land use change (4) Including diesel-powered vehicles, ethanol represented 18% of the road sector fuel consumption in 2006. (5) Brazilian ethanol production is no longer subsidized, but gasoline is heavily taxed favoring ethanol fuel consumption (~54% tax). By the end of July 2008, the average gasoline retail price in Brazil was USD 6.00 per gallon, while the average US price was USD 3.98 per gallon. The latest gasoline retail price increase in Brazil occurred in late 2005, when the oil price was at USD 60 per barrel

Ethanol in U.S. produced from maize costs 2.62$ gallon^{-1} and Brazilian cane ethanol (100%) price is 3.88$ gallon^{-1}. (Renewable Fuels Association, 2008,

Wikipedia, 2008). Many countries are interested in ethanol production as a transportation fuel instead of petroluem.

Table 1.3 depicts the top 15 countries producing ethanol as fuel and Turkey takes place in the 11. line with a 15.8 million galloon ethanol potential.

Table 1.3 Annual fuel ethanol production by countries
(Renewable Fuels Association, 2008)
.

Fuel Ethanol Production by country for a year (2007) Top 15 countries/blocks (Miilions of U.S. Liquid gallons)		
World rank	Fuel Country/Region	Ethanol Production 2007
1	United States	6,498.60
2	Brazil	5,019.20
3	European Union	570.3
4	China	486
5	Canada	211.3
6	Thailand	79.2
7	Colombia	74.9
8	India	52.8
9	Central America	39.6
10	Australia	26.4
11	Turkey	15.8
12	Pakistan	9.2
13	Peru	7.9
14	Argentina	5.2
15	Paraguay	4.7
	World Total	13,101.70

An economically viable dehydration plant needs a minimum 60,000 lt. ethanol. A feasibility report for an ethanol plant showed that operating and capital service costs of producing ethanol from whey permeate at maximum technical potential, was U.S. $0.6-0.7 per liter and 1.47 kg lactose l^{-1} ethanol is required with 100% ethanol conversion for this purpose (± 20 percent uncertainty). For every $0.01 net lactose value (price of lactose net of processor's cost), the feedstock cost for fermentation would be $0.1229 per gallon of ethanol. This price is formulated by considering

economy-of-scale effects, transportation costs, waste uses, and included assumptions listed bellow: (Ling K.C.,2008)

- Fermentation occurs at local plants. (In New Zealand U.S. $1.60-1.85 per gallon; in U.S. ±20 percent of New Zealand price)
- Operation of the plant (Labor, energy, supplies, repair and maintenance, depreciation, insurance, licensing fees, etc.; $1 per gallon)
- Distillation to 96-percent ethanol is made at local plants.
- Transportation of distillate is made to centrally located dehydration plant.
- Capital service cost per year was assumed to be ±20 percent of capital cost
- For a media that contained 3-4 percent ethanol, the ethanol recovery cost was at least $0.54 per liter

Direct fermentation of CW to ethanol yields low ethanol concentrations (2-3%, vv^{-1}) because of low lactose content and therefore, is not economical. Distillation costs for ethanol separation from dilute fermentation broths (2-3% EtOH) is a major cost item in ethanol fermentation of CW. Ultrafiltration (UF) processes have been used to concentrate lactose in cheese whey before fermentation. UF improves the lactose concentration by a factor of 5 to 6 and is expensive (approx. 50 USD/ m^3). Dry cheese whey powder (CWP) may be an attractive raw material for ethanol production. Utilization of CWP instead of CW for ethanol fermentation has considerable advantages such as elimination of costly ultrafiltration processes, compact volume, long term stability and high concentrations of lactose and other nutrients. The cost of CWP production from cheese whey by spray or drum drying varies between 20-40 cents/kg CWP which is much lower than distillation costs for pure ethanol production from dilute cheese whey. High ethanol concentrations (12-13 %, v v^{-1}) can be obtained by fermentation of concentrated CWP solutions (250 g lactose l^{-1}) to reduce the distillation costs. (Özmıhçı S. Kargı F., 2008; Siso, 1996)

The Annual Energy Outlook 2007 with projections to 2030 forecasts ethanol wholesale price for long-term trend is to be in the range of $1.650 to $1.720/gal. (Ling K.C.,2008; Renewable Fuels Association, 2008, Wikipedia, 2008)

1.9 Objectives and Scope of This Study

The objective of this study is to investigate ethanol production by fermentation of CWP and to determine the most suitable operation method and the conditions. Batch, fed -batch and continuous (suspended and fixed biofilm) operational modes were used for this purpose. Sugar utilization, ethanol and biomass formation were investigated in experimental studies.

Objectives of the proposed study can be summarized as follows:

- To determine the potential advantages of using CWP solution for ethanol fermentation as compared to cheese whey (CW) and lactose,
- To compare and select the most suitable *Kluyveromyces* strain for ethanol fermentation from CWP solution.
- To investigate the effects of major operating variables such as initial pH, external N and P additions, CWP concentration, biomass concentrations on ethanol formation using batch experiments.
- To determine sugar utilization, ethanol formation, biomass growth in fed batch operational mode at different feed CWP concentrations while the other operating parameters were constant.
- To study ethanol fermentation of cheese whey powder (CWP) solution in an agitated fermenter operated in continuous mode at different hydraulic retention time (HRT) and different feed sugar concentrations.
- To investigate the effects of hydraulic residence time (HRT) and the feed sugar content on ethanol fermentation of CWP solution in a packed column bioreactor (PCBR) filled with olive pits.

CHAPTER TWO
LITERATURE REVIEW

Ethanol fermentation from different raw materials containing carbohydrates have been studied extensively in the past (Mielenz, 2001; Hari et al., 2001; Nigam, 2001; Gong et al., 1999; Cheung and Anderson, 1997; Agu et al., 1997; Lark et al., 1997; Duff and Murrey, 1996; Zayed and Meyer, 1996; Palmqvist et al., 1996; Siso, 1996; Lightsey, 1996). Among the most widely used raw materials for ethanol fermentations are cellulosic materials (straw, baggase, waste paper), starch containing materials (corn, wheat, rice), sugar cane, sugar beet and molasses. Utilization of waste materials for ethanol formation offer special advantages by providing cheap raw materials and simultaneous waste treatment with ethanol production.

Waste biomass has been the most widely used raw material for production of ethanol (Mielenz, 2001; Hari et al., 2001; Nigam, 2001; Gong et al., 1999; Cheung and Anderson, 1997; Agu et al., 1997; Lark et al., 1997; Duff and Murray, 1996; Zayed and Meyer, 1996; Palmqvist et al., 1996). However, ethanol production from waste biomass is expensive since the process requires separation of lignin from cellulose, hydrolysis of cellulose to sugars, fermentation of sugar solution to ethanol and separation of ethanol from water. Production of ethanol from starch containing materials such as corn may be technically more feasible as compared to biomass as the raw material. However, high cost of corn and other starch containing grains makes the process economically less attractive. Among the inexpensive and highly available raw materials for ethanol production are molasses and cheese whey which are the waste by-products of sugar and dairy industries.

Whey as a high strength wastewater has to be treated before discharging to the environment. Repeated fed-batch culture of *T. cremoris* and *C. utilis*, carried out in an airlift bioreactor operating in variable volume mode is a potential alternative for the treatment of whey, with the production of high yield of biomass (0.75 g biomass g^{-1} lactose) and high yield of COD removal (95.8%) (Cristiani-Urbina et.al., 2000).

Continuous ethanol production without effluence of wastewater was investigated by Ohashi et.al. (1998) using a closed circulation system which integrated a cell retention culture system and a distillation system to separate ethanol. The stirred ceramic membrane reactor (SCMR), a jar fermentor fitted with asymmetric porous alumina ceramic membrane rods was used for retaining high density of cells and extraction of the culture supernatants that was continuously sent to the distiller to evaporate ethanol. After the distillation process, the residual solution of the culture supernatant was returned to the SCMR via a heat exchanger. When the ethanol concentration reached to 60 g l^{-1} in the fermentor, cultivated with two different *Saccharomyces cerevisia* strains the culture supernatant was extracted by filtration and sent to the distiller. During the repeated ethanol fermentation and recycling of the medium cell concentration increased to 236 g l^{-1} and productivity of ethanol reached to 13.1 g l^{-1} h^{-1}. (Ohashi et.al., 1998)

Ethanol fermentation of sugar by *Saccharomyces cerevisiae* in an immobilized cell reactor (ICR) was carried out to improve the performance of the fermentation process (Najafpour et.al., 2004). In batch fermentation, sugar consumption and final ethanol obtained were 99.6% and 12.5% v v^{-1} after 27 h while in the ICR, 88.2% and 16.7% v v^{-1} were obtained with 6 h retention time. Nearly 5% final ethanol was achieved with high glucose concentration (150 g l^{-1}) at 6 h retention time. A yield of 38% was obtained with 150 g l^{-1} glucose. The yield was improved approximately to 27% in ICR and a 24 h fermentation time was reduced to 7 h. The cell growth rate was based on the Monod rate equation. The kinetic constants; K_s and R_m of batch fermentation were 2.3 g l^{-1} and 0.35 g l^{-1} h, respectively. The maximum yield of biomass and the product formation in batch fermentation were 50.8% and 31.2%, respectively. Productivity of the ICR were 1.3, 2.3, and 2.8 g l^{-1} h for 25, 35, 50 g l^{-1} of glucose concentration, respectively. The productivity of ethanol in batch fermentation with 50 g l^{-1} glucose was calculated as 0.29 g l^{-1} h^{-1}. Maximum production of ethanol in ICR was 10 times higher as compared to suspended culture batch operation. The present research has shown that high sugar concentration (150 g l^{-1}) in the ICR column was successfully converted to ethanol. The achieved results in ICR with high substrate concentration are promising for scale up operation. (Najafpour et.al., 2004)

The production of ethanol from starch has been investigated in a genetically modified *Saccharomyces cerevisiae* strain, YPB-G, which secretes a bifunctional fusion protein that contains both the *Bacillus subtilis* α-amylase and the *Aspergillus awamori* glucoamylase activities. Fed-batch cultures with 40 g l^{-1} starch concentration produced high yields of ethanol on starch (0.46 g ethanol g^{-1} substrate) through longer production periods. (Altıntaş et.al. 2002)

Sugar compounds present in chopped solid-sweet sorghum particles were fermented to ethanol in a rotary drum fermentor with *Saccharomyces cerevisiae*. The rate of ethanol formation decreased with increasing rotational speed. The maximum rate and extent of ethanol formation were 3.1 g l^{-1} h^{-1} ethanol and 9.6 g ethanol 100 g^{-1} mesh respectively at 1 rpm rotational speed.(F. Kargi, J. Curme, 1985)

Solid state fermentation of chopped sweet sorghum particles to ethanol was studied by Kargi et.al. (1985a) in static flasks using *Saccharomyces cerevisiae*. The influence of various process parameters, such as temperature, yeast cell concentration, and moisture content, on the rate and extent of ethanol fermentation was investigated. Optimal values of these parameters were found to be 35° C, $7x10^8$ cells g^{-1} raw sorghum, and 70% moisture level, respectively.(F.Kargi et.al., 1985a)

Ghaly and El-Taweel (1997) developed a kinetic model for continuous ethanol fermentation of cheese whey. The model accounts substrate limitation, substrate inhibition, ethanol inhibition and cell death. Three bioreactors of 5 l volume were operated at different hydraulic retention times (HRT) ranging from 18 to 42 h and initial lactose concentrations ranging between 50 to 150 g l^{-1}. The experimental data were used to validate the model. The model predicted the cell, lactose and ethanol concentrations with high accuracy (R^2= 0.96-0.99). The cell concentration, lactose utilization and ethanol production were significantly affected by hydraulic retention time and the feed substrate concentration. Lactose utilizations of 98, 91 and 83% were obtained with 50, 100 and 150 g l^{-1} initial lactose concentrations at 42 h HRT. The highest cell concentration (5.5 g l^{-1}), highest ethanol concentration (58.0 g l^{-1}) and maximum ethanol yield (99.6% of theoretical) were achieved at 42 h HRT and 150 g l^{-1} initial lactose concentration. The kinetic constants found in this study were

μ_m=0.051 h^{-1}, k_d = 0.005 h^{-1}, K_s = 1.900 g l^{-1}, K_p = 20.650 g l^{-1}, K_s'= 112.510 g l^{-1}. (Ghaly, El-Taweel, 1997)

Kluyveromyces marxianus UFV-3 batch fermentations were conducted under aerobic, hypoxic, and anoxic conditions with (cheese whey permeate) initial lactose concentrations ranging between 1 and 240 g l^{-1} (Silveria et.al. 2005). Increases in lactose concentration increased ethanol yield and volumetric productivity, but reduced the cell yield. When lactose concentration was equal or above 50 g l^{-1} and the oxygen levels were low, the ethanol yield was close to its theoretical value. Maximum ethanol concentrations attained in this study were 76 and 80 g l^{-1} in hypoxic and anoxic conditions, respectively. At all oxygen levels tested a tendency for saturation of the ethanol production rate above 65 g l^{-1} lactose was observed. Ethanol production rate was also higher in anoxia. (Silveria et.al. 2005)

A kinetic analysis of *Kluyveromyces lactic* fermentation on whey is reported by Barba et al. (2001). Batch and fed- batch operations were realized in 10, 100 and 1000 l fermentors. A simple kinetic model for cell growth during batch and fed-batch operation was used. As expected, the specific growth rate was well represented by the Monod equation. Kinetic parameters were estimated by fitting the model to the experimental data. The results indicated the ability of the model to predict *K. lactic* fermentation of whey at different scales (Barba et.al., 2001).

Grba et al (2002) investigated the suitability of five different strains of yeast *Kluyveromyces marxianus* for alcoholic fermentation of deproteinized whey. The selection of yeast strains was performed at different cultivation conditions: temperature ranged between 30-37 °C, lactose concentration was between 5% and 15 % and pH varied between 4.5-5.0. Acceptable results were achieved almost with all the yeast strains (under aerobic conditions in a rotary shaker), but the best results were gained with *K. marxianus* VST 44 and ZIM 75, respectively. The optimal temperature was 34 °C for both strains. Fed-batch exeriments were also performed with *K. marxianus* at 34 °C under aerobic/anaerobic conditions with a retention time of 12/14 hours. At the end of the process the biomass yield reached to 10 g l^{-1} and the ethanol content was 7.31 %. (Grba et.al., 2002)

The increases of ethanol in the fermentation media inhibits the fermentation procedure. Kaseno et al (1998) proposed a new method of long-term fermentation with minimal wastewater generation and evaluated the effect of ethanol removal by pervaporation (PV) in ethanol fermentation to alter product inhibition. Batch, fed-batch without PV and fed batch with PV experiments were performed with glucose and immobilized baker's yeast for this purpose. A module of a hydrophobic porous membrane made of polypropylene (PP) was used. Fed-batch fermentation with or without PV was carried out for 72 hours where the feed (Q) was equal to the sum of the production (P) and drain of broth (W). Ethanol concentration was constant (50 g l^{-1}) with a removal ratio of 84.4% with PV and this value was 2 times higher then the ethanol concentration obtained without PV. Glucose conversion was 96.3 % wih a total ethanol of 780 g . 38.5% of the media was discharged as wastewater from the conventional batch process. When R was 100% which means the the reverse of inhibition constant (l/K_I) approached to zero, the effect of by-product was negligible. Only the inhibition effects of ethanol in the present media reduced ethanol productivity. (Kaseno et.al. 1998)

The enzymatic hydrolysis of lactose by a commercial enzyme from a selected strain of *Kluyveromyces fragilis* has been studied by Jurado *et.al.* (2002). The variables analyzed were, temperature (25–40 ◦C), enzyme concentration (0.1–3.0 g l^{-1}), lactose concentration (0.0278–0.208 M), and initial galactose concentration (0.0347 M). This study verified that the enzyme had similar affinity to lactose and galactose with an equilibrium semi-reactions to both the substrate and the product.(Jurado et.al., 2002)

Utilization of fed-batch operation for ethanol fermentation is very limited (Lu et al., 2003; Lukondeh et al., 2005). Lukondeh et al. (2005) investigated fed-batch fermentation of cheese whey by *Kluyveromyces marxianus* with 10–60 g l^{-1} feed lactose concentrations. An average specific growth rate (0.27 h^{-1}), biomass yield (0.38 g g^{-1}) and overall productivity (2.9 g l^{-1} h^{-1}) were obtained by fed-batch operation with DO concentrations greater then 20% of saturation. Ferrari et al. (1994) also investigated ethanol fermentation of whey permeate in a fed-batch operated reactor. With an initial lactose concentration of 100 g l^{-1} and a constant

lactose feeding rate of 18 g h^{-1}, 64 g l^{-1}ethanol concentration, 3.3 g $l^{-1}h^{-1}$ethanol productivity, 0.47 g EtOH g^{-1} lactose ethanol yield, and 0.058 g biomass g^{-1} lactose biomass yield were obtained.

There are no literature reports on fermentation of CWP solution to ethanol in a continuous suspended culture fermenter and in a packed column bioreactor. The first reports on this topic were published by Ozmihci and Kargi (2007b; 2007c; 2007d; 2007e; 2008; 2009).

Tables 2.1 and 2.2 summarize some of the studies performed with different yeast strains using different raw materials and cheese whey and compare the operational conditions.

Table 2.1 Comparison of some studies with different yeast strains and raw materials

System	Organism	pH	Retention Time	T(°C)	Medium	Agitation (rpm)	Biomass	Yield coef. ($Y_{P/S}$)	Ethanol formation	productivity	Reference
Batch	Anaerobic granular sludge	7.5	46 h	37	Lactose, cheese whey powder (CWP) and glucose (0.86–29.14 g l^{-1})	150			50 mg l^{-1} (by product of hydrogen production)		Davila-Vazquez G., 2008
Batch	*Kluyveromyces marxianus* DMKU 3-1042,	5	72 h	37	a sugar cane juice (22% total sugars)			77.5% of theoretical yield	8.7%	1.45 g $l^{-1}h^{-1}$	Limtong S., 2007
SSF	*S. cerevisiae*		24 h	37	waste mushroom log (136 mg g^{-1} glucose, 61 mg g^{-1} xylose, 2.7 mg g^{-1} galactose, 1.7 mg g^{-1} mannose and 1.3 mg g^{-1} arabinose)	180			12 g l^{-1} waste mushroom logs, normal wood 8 g l^{-1}		Lee J. Et.al, 2008
Batch	*Pichia stipitis* NRRL Y-7124.	6		30	sunflower seed hull (sugar:48 g l^{-1})	100	92-1.98 g	0.32 g g^{-1}	11 g l^{-1}	0.065 g L^{-1} h^{-}	Telli-Okur M, Eken-Saraçoğlu N., 2008
SSF	*Saccharomyces cerevisiae*	6	24 h	37	citrus peel waste (Pectinase activity:297 IUg^{-1} dry matter	10–12	0.7 g cells/100 g		39.6 g l^{-1}		Wilkins M.R . Et.al, 2007
Batch	*Zymomonas mobilis, Candida tropicalis*	6	72 h	30	enzyme hydrolized agro-industrial waste (thippi) (57.8% starch, 2% fiber, 1% protein and 3% pectin)	180	72.8 g l^{-1}	0.48 g g^{-1}	254.45 g ethanol kg^{-1} thippi		Patlea S., Lalb B.,2008
SSF	*E. coli* (KO11)	5.5	96 h	38	Barley hull, a lignocellulosic biomass,83% for glucan and 63% for xylan	150		89.4% and 88.4% of the maximum theoretical	20-26 g l^{-1}		Kim T. Et.al., 2008
semicontinuous solids-fed bioreactors ''original'' design ''retrofitted''design	Saccharomyces cerevisiea	4.5	30 days	37	paper sludge glucan (62 wt.%, dry basis), xylan (11.5%), and minerals (17%)	100 60		0.466	42 g l^{-1}		Fan Z. et.al., 2003

Table 2.2 Comparison of some studies with *K. marxianus* and/or cheese whey as raw material

System	Organism	pH	Retention Time	T(°C)	Medium	Agitation (rpm)	Biomass	Yield coef. ($Y_{P/S}$)	Ethanol formation	productivity	specific growth rate	Reference
Fed- batch	*Kluyveromyces marxianus*	4.5		30	15%(w/v) dehydrate whey when Q=180 ml h^{-1}, under 2vvm aeration	350	28.13 g l^{-1}	0.58 g g^{-1}		2.42 g l^{-1} h^{-1}	0.63 1 h^{-1}	Belem, Lee, 1998
Simultaneous saccarification and fermentation (SSF)	*Kluyveromyces marxianus*		72-82 h.	42	lignocellulosic substrates (*Populus nigra, Eucalyptus globulus*, wheat straw, sweet sorghum, herbaceous residue)			0.31-0.36 g g^{-1}	19-16g l^{-1}			M.Ballesteros et.al., 2003
Continuous	*Candida pseudotropicalis* ATCC 8619		42 h.		Cheese whey	300	3-5 gl^{-1}	0.25-0.47 g g^{-1}	20-60 g l^{-1}			Ghaly, El-Taweel, 1997
Batch	*Kluyveromyces marxianus*	5. 5	30 h.	30-42	Cheese whey	600 with airation	6 g l^{-1}	0.3-0.41 g g^{-1}			max. 0.6 h^{-1}	Longhi et.al., 2004
Batch	*Kluyveromyces marxianus*	3.8-6.1	48 h.	20-35	corn slage juice	200	13.3 g l^{-1}		8.85 g l^{-1}			Hang et.al., 2003
Batch	*Kluyveromyces marxianus*	5. 5	600-1200 s.	29	dehydrated whey and essential nutrients	700	12.2 g l^{-1}	0.4 g g^{-1}	12.3 g l^{-1}		0.35 h^{-1}	G. Cortes, 2005
Batch	*Kluyveromyces marxianus*	5	10 h.	30	sugar solution 14-26 g l^{-1}	700		0.49 g g^{-1}		0.83 g $^{-1}$ $l^{-1}h^{-1}$		Bellaver et.al., 2004
Repeated batch	*Kluyveromyces marxianus*	6 -4	72 h.	37-50	whey				9% max.			Karkoutas et.al., 2002
Fed-batch	*Kluyveromyces marxianus*	4. 5	50 h.	30	whey	350	20 g l^{-1}		1 % with oxygen			Belem & Li, 1999
Batch	*Kluyveromyces marxianus strain MTCC 1288*	4.5	22 h	34	crude whey.	500	8.9 g $l^{?1}$		2.10 g $l^{?1}$ (q_p=0.046 $h^{?1}$)		0.157 h?1	Zafar S & Owais M.,2006
semi-continuous	*S. cerevisiae co-immobilized with b-galactosidase crosslinked with glutaraldehyde.*	4.5–5.0	20-day	30	dried permeate from milk ultrafiltration lactose mash (12%)				4.56% m/v (In a cycle 6.19% was accieved)	1.3		Lewandowska M.&Kujawski W., 2007
Batch, fed-batch	*K. marxianus FII 510700 (FRR 1586)*	5	12 h 34.5 h	30	40 g l^{-1}dissolved oxygen concentrations greater than 20%		0.41 g g^{-1},15 g l^{-1}(biomass	q_s 0.66 g g^{-1} q_s 0.95g g g^{-1}	3.48% in batch	1.26g l^{-1} h^{-1} 2.9 g l^{-1} h^{-1}	0.37 h^{-1} 0.27 h^{-1}	Lukondeh T. et.al., 2005
Batch	*Kluyveromyces marxianus var. marxianus, designated IMB3*	5.8	16-18 h	45	molasses 23% (v/v).	200 rev min^{-1}			7.4% (v/v)	1 gl^{-1} h^{-1}		Gough S.et.al., 1996
continuous (airlift bioreactor)	*recombinant flocculating Saccharomyces cerevisiae*	4.0 ± 0.1.	120 h.	30 ± 1	cheese whey permeate, 50-100 g l^{-1} (dilution rate: 0.45 $h^{-1)}$	filtered air 1.0000 ± 0.0002 vvm.	42 g l^{-1}		50 g l^{-1}	10 g $l^{?1}$ $h^{?1}$		Domingues L., 2000

CHAPTER THREE
MATERIALS AND METHODS

3.1 Batch Experiments

3.1.1 Experimental System

Batch experiments were performed by using sterile erlenmeyer flasks and a gyratory shaker. The erlenmeyer flasks were prepared in dublicates, sterilized at 121 ^{o}C for 20 minutes and inoculated with 20 ml pure *Kluyveromyces marxianus* cultures and 200 mg l^{-1} Na-thioglycolate as the reducing agent (200 ml total volume). Inoculated flasks were placed on a gyratory shaker at 28 ± 2 ^{o}C and 100 rpm. The initial pH of the media was adjusted to 5. Samples were withdrawn aseptically from the experimental flasks periodically for analysis of total sugar and ethanol. A control flask free of yeast cells containing various CWP and 200 mg l^{-1} Na-thioglycolate was used to determine any ethanol formation or sugar utilization in the absence of yeast cells.

3.1.2 Experimental Procedure

3.1.2.1 Comparison of Different Substrates

In selection of the most suitable substrate for ethanol formation cheese whey (CW), cheese whey powder (CWP) and lactose solutions were used as substrate with an initial total sugar concentration of 28 g l^{-1}. Compositions of the CW and CWP used are summarized in Table 3.1. NH_4Cl (1.538 g l^{-1}) and KH_2PO_4 (1.63 g l^{-1}) was added to the flasks containing lactose to obtain C/N/P ratio of 100/3/1.5. Dublicate erlenmeyer flasks (500 ml) were charged with 180 ml of deionized water containing 104 g l^{-1}CWP (50 g l^{-1} total sugar) and 200 mg l^{-1} Na-thioglycolate as the reducing agent. 20 ml of pure *Kluyveromyces marxianus* strains (*Kluyveromyces marxianus* NRRL-1109 and NRRL-1195) were used for inoculation of the erlenmeyer flasks after sterilization.

Table 3.11. Typical composition of cheese whey and Cheese whey powder used in the experiments. (CWP=10 g l^{-1} in CWP solution). Concentrations are in mg l^{-1}.

	T-Sugar	*T-COD*	*S-COD*	*T-TOC*	*S-TOC*	*SS*	*TN*	*TP*	*Fat*	*pH*
CW	28000	59800	42260	28848	21588	1869	≈2000	900	545	4.4
CWP Soln.	5100	11400	8800	3900	3300	100	306	156	260	6.2

T-sugar: total sugar; T-COD: total chemical oxygen demand; S-COD: soluble chemical oxygen demand; T-TOC: total organic carbon; S-TOC: soluble total organic carbon; SS: suspended solids in mg l^{-1}, TN: Total Nitrogen, TP: Total Phosphorus.

3.1.2.2 Selection of Organism

In these experiments three yeast strains (*Kluyveromyces marxianus* NRRL-1109, NRRL-1195 and DSMZ 7239) were compared for their sugar utilization and ethanol formation capabilities from CWP solution. Dublicate erlenmeyer flasks (500 ml) were charged with 180 ml of deionized water containing 104 g l^{-1}CWP (50 g l^{-1} total sugar) and 200 mg l^{-1} Na-thioglycolate as the reducing agent and 20 ml of pure *Kluyveromyces marxianus* strains were inoculated to the erlenmeyer flasks after sterilization.

3.1.2.3 Effects Of Operating Conditions

Five hundred ml erlenmeyer flasks were charged with 180 ml of deionized water containing desired concentrations of CWP between 52 and 312 g l^{-1}. 200 mg l^{-1} Na-thioglycolate as the reducing agent and 20 ml of yeast strain (*K. marxianus* NRRL-1195) were added to the flasks. The initial pH of the media was adjusted to desired level between pH 3 and 7 in variable pH experiments. Initial pH was 5 in other experiments.

Five different flasks were prepared to find out the most suitable initial ORP value for *K.marxianus* DSMZ-7239. ORP was adjusted by adding different Na-thioglycolate concentrations to the flasks. Na-thioglycolate concentrations varied between 50- 300 mg l^{-1} to obtain ORP's between -20- -163 mV. A control flask free of yeast cells containing 52 g l^{-1} CWP and 200 mg l^{-1} Na-thioglycolate was used to determine any ethanol formation or sugar utilization in the absence of yeast cells.

3.1.2.4 Effects of External Nutrient Additions

In order to determine if CWP is nutritionally balanced for ethanol fermentation NH_4Cl and KH_2PO_4 salts were added to the 52 g l^{-1}CWP solution (approx. 25 g l^{-1} sugar) and the yields of ethanol formation were evaluated with *K. marxianus* NRRL-1195. Seven different experiments were performed with different N and P contents. In the two experimental flasks the N content of CWP was increased twice and four times by external addition of NH_4Cl while the phosphorous content was constant. In the other two flasks P content of CWP was increased twice and four times while the nitrogen content was constant. The last two flasks contained doubled or quadrupled N and P with external additions.

3.1.2.5 Experiments with Different CWP and Yeast Concentrations

In variable substrate (CWP) concentration experiments, the erlenmeyer flasks (500 ml) were charged with 180 ml of deionized water containing desired concentrations of CWP between 52 and 312 g l^{-1} and 200 mg l^{-1} Na-thioglycolate as the reducing agent. The erlenmeyer flasks were inoculated with 20 ml pure *Kluyveromyces marxianus* NRRL-1195 and DSMZ-7239 culture, respectively (200 ml total volume). Variable biomass concentration experiments were performed by inoculating the experimental flasks with different amounts of inoculum culture by using *K. marxianus* DSMZ-7239 (10-60 ml) and CWP solution (190-140 ml) to obtain a total volume of 200 ml in every flask.

3.1.3 Organisms

Kluyveromyces marxianus strains of NRRL-1109, NRRL-1195 were obtained from USDA Northern Regional Research Laboratories, Peoria, Ill, USA; and *Kluyveromyces marxianus* DSMZ-7239 from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in lyophilized form. The yeast strains were cultivated in laboratory using an incubator shaker under sterile conditions at pH 5, 28 ^{o}C, 100 rpm for 5 days. Pure cultures grown anaerobically were used for inoculation of experimental systems.

3.1.4 Medium Composition

Medium used for cultivation of inoculum culture consisted of yeast extract (5 g l^{-1}), peptone (5 g l^{-1}), NH_4Cl (2 g l^{-1}), $KH_2 PO_4$ (1 g l^{-1}), $MgSO_4$. $7H_2O$ (0.3 g l^{-1}), lactose (30 g l^{-1}) and 200 mg l^{-1} Na-thioglycolate as the reducing agent at pH =5. The initial oxidation-reduction potential (ORP) of the media was nearly -250 mV indicating the anaerobic conditions. The yeast culture grown on a shaker in the aforementioned media at 28 ^oC and 100 rpm was used for inoculation.

The cheese whey powder (CWP) was obtained from Pınar Dairy Industry in Izmir, Turkey and was dried at 80 ^oC before use. The CWP contained approximately 49% (w w^{-1}) total sugar, 20% protein, 2.6% fats, 3% total nitrogen and 0.96% total phosphorous on dry weight basis. (Table 3.1.1)

The experimental flasks contained desired concentrations of CWP and 200 mg l^{-1} Na-thioglycolate (ORP = - 250 mV) in deionized water at pH =5.

3.1.4.1 Comparison Of Different Substrates

Cheese whey and CWP solutions were used without addition of any external nutrients with an initial total sugar concentration of 25 g l^{-1}. Lactose solution contained 28 g l^{-1} lactose, 1.54 g l^{-1} NH_4Cl and 1.66 g l^{-1} $KH_2 PO_4$.

3.1.4.2 Performance of different K. marxianus strains in CWP fermentation

Dublicate erlenmeyer flasks (500 ml) were charged with 180 ml of deionized water containing 104 g l^{-1} CWP (50 g l^{-1} total sugar).

3.1.4.3 Effects Of Operating Conditions

Initial CWP concentrations were 70 g l^{-1} , 50 g l^{-1} and 50 g l^{-1} in variable pH, ORP and external nutrient addition experiments, respectively. When N and P contents of CWP solution were doubled, 5.8. g l^{-1} NH_4Cl and 2.1 g l^{-1} KH_2PO_4 were added to the 50 g l^{-1} CWP solution. CWP concentration was varied between 52 and 312 g l^{-1} in variable CWP experiments without any external N and P additions. In ORP experiments; ORP was adjusted by addition of different Na-thioglycolate

concentrations to the experimental flasks. 50, 100, 200, 250 and 300 mg l^{-1} Na-thioglycolate concentrations were added to obtain -20, -80, -140, -158, -163 mV ORP's, respectively.

3.1.4.4 Experiments With Different CWP And Yeast Concentrations

CWP concentration was varied between 52 g l^{-1} and 312 g l^{-1} in variable CWP experiments which correspond to nearly 26 and 156 g l^{-1} soluble sugar concentrations. Total soluble sugar concentration was almost 50% of the CWP concentration. In variable inoculum size experiments the initial biomass concentration was varied between 170 and 1020 mg l^{-1} while the CWP concentration was constant at 100 g l^{-1}.

3.1.5 Analytical Methods

The samples were removed from the flasks periodically and centrifuged at 8000 rpm (7000 g) to remove solids from the liquid media. Analyses were carried out on the supernatants after centrifugation. Total reducing sugar concentrations were measured by using the phenol-acid method (Dubois *et al.* 1956). The samples were analyzed in triplicates and results were reproducible within 3% deviation. Ethanol concentrations were measured using a Gas Chromatograph (Varian CP–3800) with an FID dedector and a WCOT fused silica capillary column (15mx 0.25 mm ID, 0.25µm film thickness). The column temperature was set for 75 ^{o}C for 1 min and raised to 130 ^{o}C with a rate of 20 ^{o}C/min yielding a total hold time of 4.75 min. Temperatures of injector and dedector were 150 ^{o}C and 200 ^{o}C, respectively. Nitrogen was used as the carrier gas with a linear velocity of 25 ml min^{-1}.

Oxidation reduction potentials (ORP) and pH were measured using a pH meter (WTW) with either an ORP or a pH probe. Biomass concentrations were determined

by filtering the samples through 0.45 µm milipore filter papers and drying at 105 ^{o}C until constant weight.

3.2 Experiments with Fed–Batch Operation

3.2.1 Experimental System

Fed-batch experiments were performed by using a 5 liter fermenter (New Brunswick, model IIC) as shown in Figure 3.1. The operation was started batch wise with sterile CWP solution and the fermenter was inoculated with pure culture of *K. marxianus* DSMZ-7239. The batch operation was repeated several times with biomass sedimentation and supernatant removal at the end of every batch operation until highly dense biomass concentration was obtained. Fed-batch operation was started with a highly dense culture volume of 1 liter. Sterilized feed CWP solution was kept in a refrigerator at 4 ^{o}C to avoid any decomposition and was fed to the reactor under aseptic conditions with a flow rate of 0.084 l h^{-1} by using a peristaltic pump (Watson-Marlow model 323). Samples were withdrawn from the fermenter aseptically every hour for pH, ORP, total sugar, biomass (total suspended solids) and ethanol measurements. Na-thioglycolate (200 mg l^{-1}) was added to the CWP solution in order to adjust the ORP to lower than -200mV. Agitation speed was 100 rpm with N_2 gas passage through the fermenter for 15 minutes every day. pH of feed CWP solution was adjusted to 5 before sterilization. pH of the fermentation media varied between 4 and 4.5 during operation while the temperature was 26 ± 2 ^{o}C. Each fed-batch cycle continued for 48 hours with agitation (100 rpm) followed by 24 hours of batch operation without agitation to reduce the sugar content below 1 g l^{-1} at the end of each cycle. Three liters of the fermenter contents was removed at the end of each cycle and the next fed-batch cycle was started with the 2 liter initial volume and a flow rate of 0.084 l h^{-1}. Repeated fed-batch operations were performed for five cycles where the system reached the quasi steady-state. Control fed-batch experiments were performed under the same conditions as that of the actual experiments in the absence of yeast cells to quantify sugar concentrations without fermentation.

3.2.2 Organisms

Kluyveromyces marxianus DSMZ–7239 was used in the experiments and was prepared as explained in part 3.1.3 The inoculum culture was prepared by inoculating 180 ml sterile CWP (50 g l^{-1}) solution by 20ml of the pure yeast strain from a liquid culture. The culture was grown in an incubator gyratory shaker, at 100 rpm and at 28^{o}C for 5 days. Then, five erlenmeyer flasks, containing adapted *Kluyveromyces marxianus* culture with a total volume of 1 l were used for inoculation of the fermenter.

3.2.3 Medium Composition

The growth medium of the yeast strain was explained in part 3.1.4 . The feed media used for the fed-batch experiments contained desired concentrations of CWP and 200 mg l^{-1} Na-thioglycolate (ORP = - 250 mV) in deionized water at pH 5. Feed CWP concentrations varied approximately between 51 g l^{-1} and 408 g l^{-1} in fed-batch experiments yielding nearly 25±1 and 200±10 g l^{-1} soluble sugar since sugar concentrations were approximately 49% of CWP. Feed CWP solution was heated to 90^{o}C for deproteinization, the solids were removed and the supernatant was autoclaved at 121^{o}C for 20 min for sterilization. Sterilized feed CWP solution was kept in a refrigerator at 4 ^{o}C to avoid any decomposition.

3.2.4 Analytical Methods

The procedure was the same as in batch eperiments explained in part 3.1.5 . For biomass concentration total suspended solids (TSS) were also determined by drying 10 ml samples from the feed and the reactor at 105 ^{o}C until constant weight. Difference in total suspended solids content of the fermenter and the feed was considered as the biomass yield during fermentation.

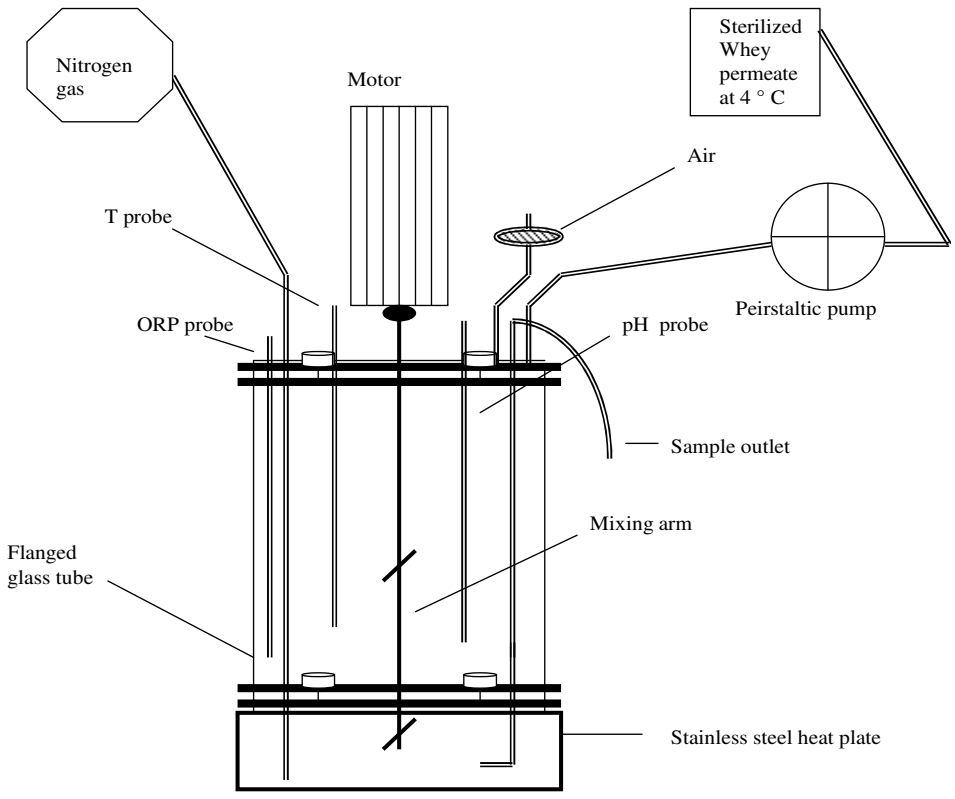


Figure 3.1 Schematic diagram of fermenter used in fed-batch and continuous experiments

3.3 Experiments with Continuous Operation

3.3.1 Experimental System

Continuous experiments were performed by using a 5 litre fermenter (New Brunswick, Model IIC) depicted in Figure 3.1. The operation was started batch-wise with sterile CWP solution (100 g l^{-1} sugar) inoculated by pure culture of *K. marxianus* DSMZ 7239. The batch operation continued until total sugar was below 20 g l^{-1} and then the continuous operation was started by feeding the CWP solution to the fermenter with a desired flow rate. The volume of the fermentation media in the fermenter was 3 litre. The HRT was varied by changing the feed flow rate. Sterilized feed CWP solution was kept in a refrigerator at 4 ^{o}C to avoid any decomposition and was fed to the reactor under aseptic conditions with a desired flow rate using a peristaltic pump (Watson-Marlow model 323, UK). Samples were withdrawn from

the fermenter aseptically every day for pH, ORP, total sugar, biomass (total suspended solids) and ethanol measurements. Na-thioglycolate (200 mg l^{-1}) was added to the CWP solution in order to adjust the ORP to lower than -200mV. Agitation speed was 100 rpm with N_2 gas passage through the fermenter for 15 minutes every day. pH of feed CWP solution was adjusted to 5 before sterilization. pH of the fermentation media varied between 4 and 4.5 during operation while the temperature was 28 ± 1 ^oC. Every continuous operation lasted until the system reached the steady-state with approximately the same sugar, ethanol and biomass concentrations in the fermenter (or in the effluent) for the last four days. Control experiments were performed in the absence of yeast cells to determine non-biological sugar utilization under the same experimental conditions as that of the actual experiments.

In experiments performed for different HRTs every experiment lasted about 6 to 10 HRT (125-600 h). Continuous experiments were performed at seven (7) different HRT levels between 12.5 and 60 hours which were established by changing the feed flow rate while keeping the fermentation volume at 3 litre constant level.

In experiments performed for different feed sugar concentration every experiment lasted about 8 to 10 HRT (430-540 h). Continuous experiments were performed at six different feed sugar concentrations between 55 and 200 g l^{-1} at a constant HRT of 54 hours.

3.3.2 Organisms

The organisms used for continuous experiments were the same as in fed- batch experiments as explained in part 3.2.2 .

3.3.3 Medium Composition

The medium composition used in continuous experiments were the same as in fed- batch experiments as explained in part 3.2.3

3.3.4 Analytical Methods

The analytical methods used were the same as the previous studies and are explained in part 3.2.4 .

3.4 Continuous Packed Column Biofilm Reactor (PCBR)

3.4.1 Experimental System and Operation

Experiments were performed using a packed column biofilm reactor (PCBR) containing olive pits as support particles. A schematic diagram of the experimental set-up is depicted in Figure 3.2, which consisted of a feed reservoir, a stainless steel PCBR operated in up-flow mode and an effluent reservoir. The feed reservoir was kept in a deep refrigerator at 4 ^{o}C to avoid decomposition of CWP solution. The column had perforated plates at the bottom and at the top to separate the particles from the liquid phase. The packed section of the column had inner and outer diameters of D_i = 8.0 cm and D_o = 9.2 cm and a height of 34.0 cm with an empty volume of 1.71 l. The PCBR contained 1920 olive pits with total particle volume of 0.92 l. The void fraction in the packed column was 0.46 with a void volume of 0.79 l. Total biofilm surface area in the column was 0.569 m^2 yielding specific surface area of 333 m^2 m^{-3} empty column or 720 m^2 m^{-3} liquid in the packed column. The column had an enlarged section at the top with an inner diameter of 10.9 cm and a height of 12 cm. The liquid volume in the enlarged section was 0.8 l with a height of 9 cm. The conical section at the bottom of the column contained fermentation broth with a volume of 0.2 l. Total liquid volume in the reactor was 1.79 l including the packed, enlarged and conical sections. The column was fed from the bottom with a desired flow rate using a peristaltic pump (Watson Marlow Model 323, Germany). The effluent was removed from the top of the column with the same flow rate by gravitational flow.

The operation was started batch-wise with medium recirculation through the column. The column was filled with sterile CWP solution (50 g l^{-1} sugar), inoculated with a dense (approx. 5 g l^{-1}) culture of *K. marxianus* (DSMZ 7239) and the medium was circulated until sugar was depleted. This procedure was repeated three times

(total of 15 days) for biofilm formation on support particles. Continuous operation was started after biofilm formation and continued until the system reached the steady-state with the same effluent sugar and ethanol concentrations, which took nearly three weeks for every experiment.

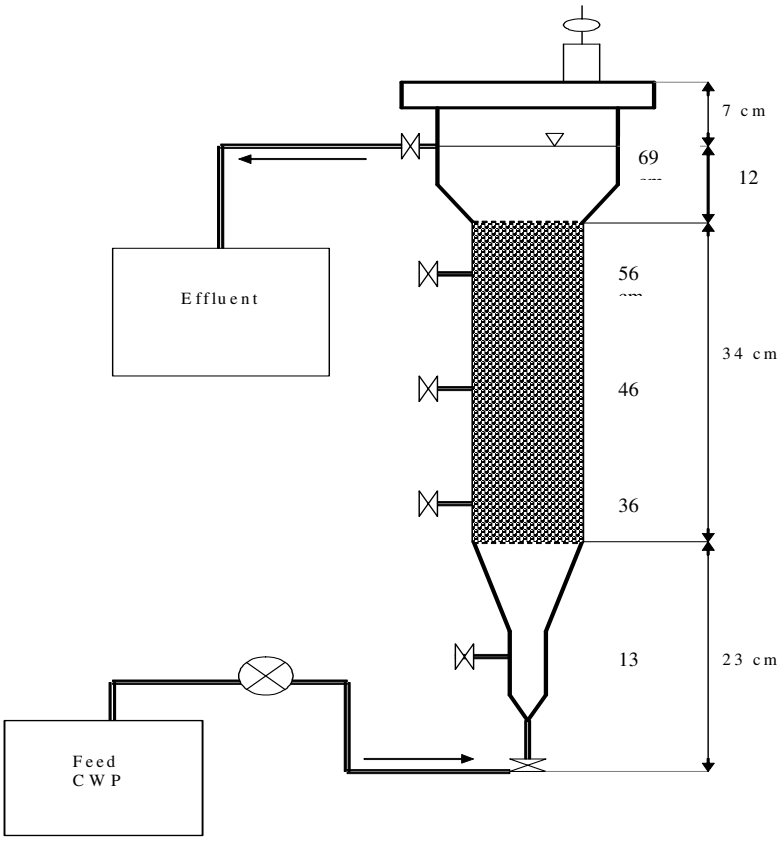


Figure 3.2 A schematic diagram of the experimental set-up

The HRT based on total liquid volume in the reactor (1.79 l) was varied between 17.6-64.4 h by changing the feed flow rate in the experiments with variable of HRT.

When the effects of the feed CWP concentration was investigated, the HRT based on total liquid volume in the reactor, (1.79 l) was kept constant at 50 h by using a

feed flow rate of 36 ml h^{-1} and the feed CWP was changed. The total sugar content changed between 50- 200 g l^{-1} in the CWP experiments.

Samples were withdrawn aseptically from the sampling ports at different heights of the column and were analyzed for sugar, ethanol and suspended biomass concentrations everyday. Temperature, pH, oxidation-reduction potential (ORP) were monitored during the course of experiments. Temperature was between 25-28 ^{o}C, pH varied between 4.3-4.6 and the ORP was between -150- and -250 mV.

3.4.2 Organisms

The organisms used in PCBR experiments were the same as fed- batch and continuous experiments described in part 3.2.2 .

3.4.3 Medium Composition

The medium composition used in these experiments were the same as explained in part 3.2.3 .

3.4.4 Analytical Methods

The samples were removed from the feed, effluent and the sampling ports at different heights of the column everyday. The analytical methods used were the same as explained in part 3.2.4

The attached biomass (biofilm) concentrations were determined by removing nearly 20 support particles from the column, washing the particles with pure water and determining the biomass concentrations by filtering and drying as described above for every experiment at the steady-state. Difference in suspended solids contents of the samples withdrawn from the column and the feed was considered as the suspended biomass concentration. Nearly 55 % of the total biomass was attached onto support particles in form of biofilm and 45 % was in suspension. Biomass and sugar concentrations decreased with the height of the column since the column was fed from the bottom. .

CHAPTER FOUR
THEORETICAL BACKGROUND

4.1 Batch Experiments

4.1.1 Kinetic Modelling and Estimation of the KineticCconstants

The following kinetic model was used to describe the initial rate of sugar (substrate) utilization for batch fermentation of CWP to ethanol by *K. marxianus* DSMZ-7239.

$$R_{SO} = \frac{k X_o S_o}{K_S + S_o} \cdot \frac{K_{SI}}{K_{SI} + S_o} \qquad \text{(Eqn 1)}$$

where R_{SO} is the initial rate of sugar utilization (g S l^{-1} h^{-1}); X_o and S_o are the initial biomass and the substrate (sugar) concentrations (g l^{-1}); k is the rate constant for sugar utilization (g S gX^{-1} h^{-1}); K_S is the saturation constant (g l^{-1}); and K_{SI} is the substrate inhibition constant (g l^{-1}).

The first term on the right hand side of Eqn 1 represents sugar utilization rate at low sugar concentrations according to the Monod equation and the second term represents substrate (sugar) inhibition at high sugar concentrations.

According to the data presented in Figure 5.18 a, sugar utilization rate increased with sugar concentration up to 78 g l^{-1} (CWP 156 g l^{-1}) and then decreased for greater sugar concentrations due to substrate inhibition. For sugar concentrations below 78 g l^{-1}, the inhibition term in Eqn 1 can be neglected and the Eqn 1 takes the following form.

$$R_{SO} = \frac{k X_o S_o}{Ks + So} = \frac{R_m S_o}{Ks + So} \qquad \text{(Eqn 2)}$$

where R_m (= kX_o) is the maximum rate of substrate utilization (g S l^{-1} h^{-1}) In double reciprocal form Eqn 2 takes the following form

$$\frac{1}{R_{SO}} = \frac{1}{R_m} + \frac{K_S}{R_m}\frac{1}{S_o} \qquad \text{(Eqn 2 a)}$$

A plot of $1/ R_{SO}$ versus $1/ S_o$ yields a line with a slope of K_S / R_m and y-axis intercept of $1/ \mu_m$.

4.2 Repeated Fed Batch Experiments

4.2.1 Calculation Methods of Repeated Fed Batch Operation

The cheese whey powder (CWP) concentration was varied between 104 and 416 g l^{-1} (Total soluble sugar (TS) = 100-200 g l^{-1}) in order to determine the effects of initial CWP or sugar concentration on the rate and extent of ethanol formation.

Theory of fed-batch operation is presented in many texts (Echegaray O.F. et.al., 2000) and is briefly summarized below. As the feed wastewater is added slowly, the liquid volume in the fermentor increases with time linearly according to the following equation since no effluent is removed:

$$V = V_0 + Q\,t \qquad \text{(Eqn 3)}$$

By controlled addition of feed, the substrate concentration remains at a low level in the fermentor named 'Quasi Steady-State' at which approximately dS/dt = 0, dX/dt = 0 and dP/dt=0. At quasi steady-state:

$$\mu = D = \frac{1}{\theta_H} = \frac{\mu_m S}{K_s + S} \qquad \text{(Eqn 4)}$$

or

$$S = \frac{K_s D}{\mu_m - D} \qquad \text{(Eqn 4 a)}$$

where D is the dilution rate ($Q/V = 1/\theta_H$). As a result of increase in reaction volume, dilution rate (D = Q/V) decreases with time in this type of operation resulting in a decrease in specific growth rate (μ) and substrate concentration. Biomass

concentration (*X*) remains almost constant; however, total amount of biomass (X_T = *XV*) in the reactor increases as a function of time according to the following equation:

$$X_T = X_{T0} + Q * Y (S_0 - S) * t \qquad \text{(Eqn 5)}$$

where *Y* is the growth yield coefficient (g *X*/g *S*), S_0 is the feed substrate concentration (g S l^{-1}) and Q is the flow rate (l h^{-1}).

4.3 Continuous Fermentor Experiments

4.3.1 Kinetic Modelling and Estimation of the Kinetic Constants

In the presence of basal (endogenous) metabolism and product formation, biomass balance in continuous fermentation yields the following equation [Shuler and Kargi, 2002; Bailey et.al. 1986; Oliveire et.al. 1999 a; Oliveira et.al. 1999 b)

$$dX/dt = DX_o + (\mu_g - b - D) X \qquad \text{(Eqn 6)}$$

where X and X_o are the biomass concentrations in the fermenter and in the feed, respectively (g l^{-1}); D is the dilution rate (Q/V, h^{-1}); μ_g is the specific growth rate (h^{-1}); 'b' is the endogenous or basal metabolism rate constant (h^{-1}).

Eqn 6 takes the following form at steady-state (dX/dt = 0), and with the sterile feed (X_o= 0).

$$\mu_N = \mu_g - b = \frac{\mu_m S}{K_s + S} - b = D \qquad \text{(Eqn 6 a)}$$

where, μ_N is the net specific growth rate (h^{-1}); μ_m is the maximum specific growth rate (h^{-1}); K_s is the saturation constant (g l^{-1}); and S is the rate limiting substrate concentration in the continuous fermenter at the steady-state (g l^{-1}). Eqn 6 a can be further arranged as follows:

$$\mu_g = \frac{\mu_m S}{K_s + S} = b + D \qquad \text{(Eqn 6 b)}$$

or in double-reciprocal form eqn 6 b can be written as:

$$1 / (D+b) = 1/\mu_m + (K_s/\mu_m)(1/S) \qquad \text{(Eqn 6 c)}$$

A plot of 1/ (D+b) versus 1/S yields a line with a slope of K_s / μ_m and y-axis intercept of $1 / \mu_m$. At high growth rates (low HRT or high dilution rates) the basal metabolism constant (b) is usually negligible.

Similarly a material balance for the rate limiting substrate (total sugar in this case) around a continuous fermenter yields the following equations.

$$dS/dt = D(S_o - S) - \mu_g X / Y_M - q_p X / Y_{p/s} \qquad \text{(Eqn 7)}$$

where, Y_M is the maximum growth yield coefficient ($Y_{x/s,M}$, gX g^{-1}S); q_p is the specific rate of product (ethanol) formation (gP g^{-1}X h^{-1}) and $Y_{p/s}$ is the product yield coefficient (gP g^{-1}S).

Eqn 7 takes the following form at the steady-state since dS/dt = 0 ,

$$D (S_o - S) = \mu_g X / Y_M + q_p X / Y_{p/s} \qquad \text{(Eqn 7 a)}$$

Since ethanol is a growth associated product, $q_p = \alpha \mu_N = \alpha D$, and $\mu_g = D + b$, then Eqn 7 a can be written as:

$$D (S_o - S) / X = q_S = (D + b)/ Y_M + \alpha D / Y_{p/s} \qquad \text{(Eqn 7 b)}$$

or

$$(S_o - S) / X = 1/Y_o = (1 + b / D)(1/Y_M) + \alpha / Y_{p/s} \qquad \text{(Eqn 7 c)}$$

where $Y_o = X/ (S_o - S)$ is the observed growth yield coefficient ($gX\ g^{-1}S$); q_s is the specific rate of substrate consumption ($g\ S\ g^{-1}X\ h^{-1}$) and α is the $Y_{P/X}$ or the amount of product formed per unit biomass formation ($gP\ g^{-1}X$).

A plot of $1/Y_o$ versus 1/D (or HRT) yields a straight line with a slope of 'b/Y_M' and a y-axis intercept of $(1/Y_M + \alpha / Y_{p/s})$.

Eqn 7 b can be solved for X and may be written as follows,

$$X = Y_M (S_o - S) (D/ (D + b + (\alpha D Y_M / Y_{p/s})) \qquad \text{(Eqn 7 d)}$$

Similar balance for the product (ethanol) formation in a continuous fermenter can be written as follows:

$$dP/dt = D (P_o - P) + q_p X \qquad \text{(Eqn 8)}$$

where, P_o and P are the product (ethanol) concentrations in the feed and in the effluent (or in the fermenter) at steady-state. Eqn 8 takes the following form at the steady state ($dP/dt = 0$) and with the product (ethanol)-free feed ($P_o = 0$)

$$DP = q_p X \qquad \text{(Eqn 8 a)}$$

Since $q_p = \alpha \mu_N = \alpha D$, then Eqn 8 a becomes

$$DP = \alpha D X \quad \text{or} \quad P/ X = \alpha \qquad \text{(Eqn 8 b)}$$

A plot of P versus X at steady-state yields a straight line with a slope of α or $Y_{P/X}$ since X_o and P_o are zero.

4.3.2 Calculation Methods for Continuous Operation

Total amount of sugar utilization, ethanol and biomass formation in continuous experiments were calculated using the following equations:

$$\Delta S = S_o - S_e$$

$$\Delta P = P_e - P_o$$

$\Delta X = X_e - X_o$

where ΔS, ΔP, ΔX are the total amount of sugar (substrate) utilized, ethanol (product) and the biomass (yeasts) produced for every operation (g l^{-1}); S_o, P_o and X_o are the feed sugar, ethanol and biomass concentrations (g l^{-1}); S_e, P_e and X_e are the effluent or the reactor sugar, ethanol and biomass concentrations at the steady-state for every operation (g l^{-1});

The yield coefficients, $Y_{P/S}$ (gP g^{-1}S) and $Y_{X/S}$ (gX g^{-1}S) as depicted in Eqn 9 and Eqn 10 were calculated by using the following equations for every HRT and feed sugar concentration.

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \qquad \text{(Eqn 9)}$$

$$Yx_{/S} = \frac{\Delta X}{\Delta S} \qquad \text{(Eqn 10)}$$

4.4 Continuous Packed Column Bioreactor (PCBR)

4.4.1 Mathematical Modeling

PCBR operating in up-flow mode behaves like a plug-flow reactor with no back-mixing at low feed flow rates (36 ml h^{-1}). Substrate balance over a differential volume $dV = A_o\, dZ$ yields the following equation when the yeast growth is negligible.

$$-Q\, dS = \frac{q_p X}{Y_{p/s}}\, dV = \frac{q_p X}{Y_{p/s}} A_o\, dZ \qquad \text{(Eqn 11)}$$

where, Q is the flow rate of the feed PWS solution (l h^{-1}); dS is the differential difference in the sugar concentration over the differential volume (g l^{-1}); q_p is the specific rate of sugar utilization (gS g^{-1}X h^{-1}); X is the average biomass concentration (g l^{-1}); $Y_{p/s}$ is the product (ethanol) yield coefficient (gE g^{-1}S); and dV is the differential volume (l), A_o is the cross section area of the column (m^2) and Z is the column height from the entrance (m). Assuming q_p, X and $Y_{p/s}$ are approximately constant, Eqn 11 can be integrated to yield the following Eqn.

$$S = S_o - \frac{q_p X}{Y_{p/s}} \theta_H = So - \frac{q_p X}{Y_{p/s}} \frac{A_o Z}{Q} \qquad \text{(Eqn 12)}$$

where, S_o and S are the sugar concentrations in the feed and at the column height of Z (g l^{-1}); θ_H is the hydraulic residence time at a certain point in the column (= V/Q = A_o Z/Q, h). A plot of sugar concentration (S) versus θ_H or column height (Z) should yield a straight line if q_p, $Y_{p/s}$ and X are constant.

Similarly, product (ethanol) balance over a differential volume dV yields the following equation:

$$Q\, dP = q_p X\, dV = q_p X A_o\, dZ \qquad \text{(Eqn 13)}$$

where, dP is the differential difference in product concentration over the diferential volume (gP l^{-1}). Integration of Eqn 13 yields the following equation.

$$P = P_o + q_p X \theta_H = P_o + q_p X (A_o Z / Q) \qquad \text{(Eqn 14)}$$

A plot of product concentration (P) versus θ_H or Z would yield a line if q_p and X are constant.

CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Batch Shake Flask Experiments

5.1.1 Comparison Of Different Substrates

Three different media were used for selection of the most suitable one by using the *K.marxianus* strains of NRRL-1109 and NRRL-1195. Lactose, cheese whey and cheese whey powder were used with an initial sugar concentration of 25 g l^{-1} in batch experiments. Experiments were performed at pH 5 with an incubation time of 72 h. The initial ORP was adjusted to < -250 mV with 200 mg l^{-1} Na- thioglycolate. Figure 5.1 depicts comparison of performances of the two strains on different substrates. Figure 5.1a shows variation of total sugar (TS) concentration with time for different media. Total sugar concentration decreased with time and the fermentation was completed in 24 hours in all experiments. Total sugar consumption was slower for the NRRL-1109 strain with CWP, which reached the others in 24 hours. Time course of variations of percent ethanol (v v^{-1}) concentrations are depicted in Figure 5.1 b Ethanol concentration in solution increased with time and reached the maximum level after 72 hours. Final ethanol concentration reached the highest level (1.8%) in 48 hours for both strains when CWP was used. Ethanol formation from CW reached its maximum level after 24 hours (1.2 %). Variations of media pH with time are depicted in Figure 5.1c. In the experiments performed with lactose, pH dropped from 5 to 3.6-3.2 in 7 hours and was stable till the end of the incubation time. pH stabilized at 4.8 with CW and 4.6 with CWP in 7 hours. ORP of the media increased with time as presented in Figure 5.1 d. ORP values increased from -275 ± 25 mV to approximately -100 mV for all experiments at the end of 72 h fermentation period.

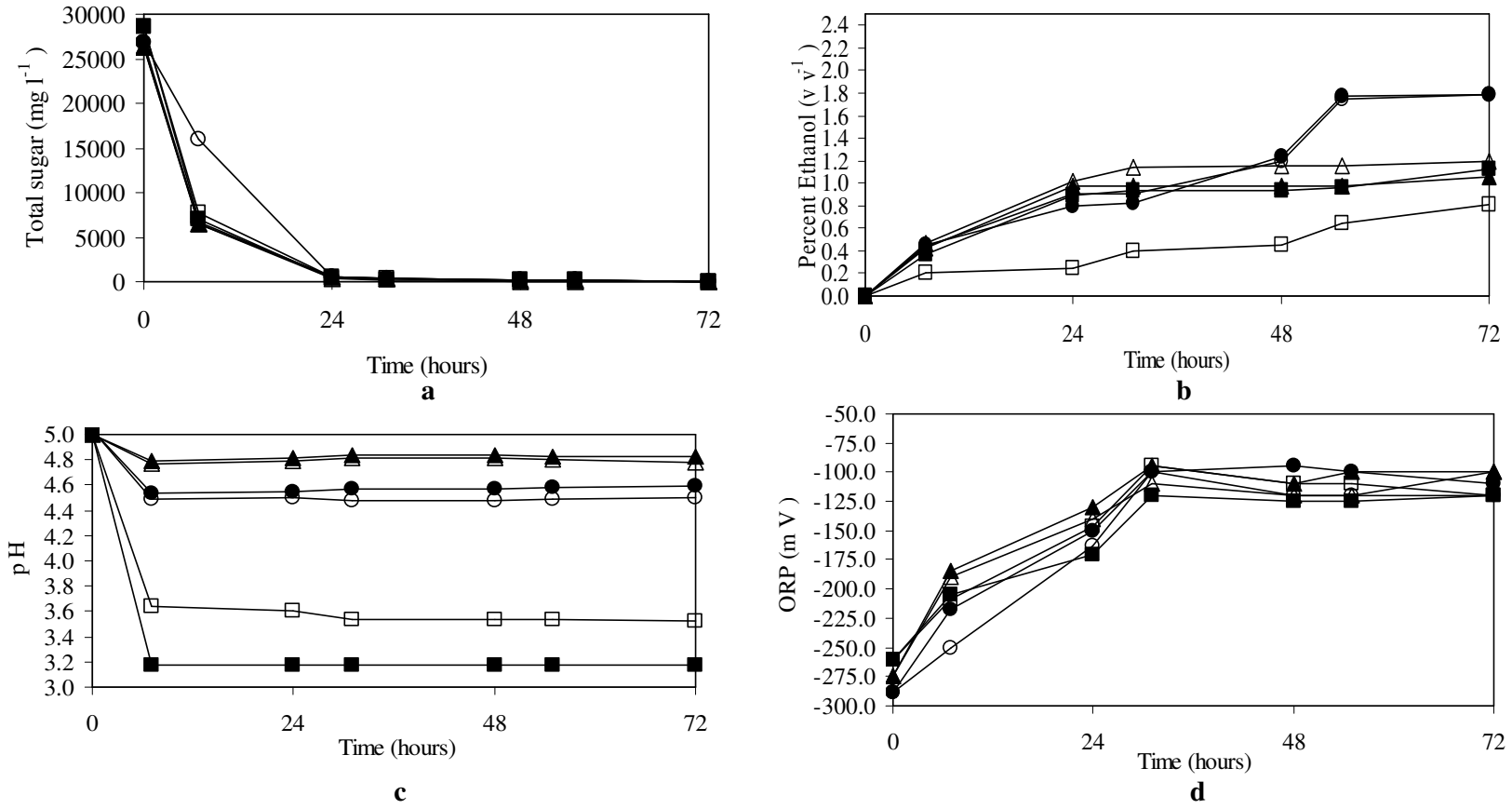


Figure 5.1 Comparison of NRRL 1109 with NRRL 1195 in different media: **a.** Variation of sugar concentration with time **b.** Variation of percent ethanol with time **c.** Variation of pH with time **d.** Variation of ORP with time. ○ CWP 1109● CWP 1195, □ Lactose 1109, ■ Lactose 1195,▲ CW 1195, Δ CW 1109

Figure 5.2 a. depicts variation of final ethanol concentration with different media and strains. The maximum ethanol yield was obtained with CWP media where performances of both strains were the same (1.8% ethanol, v v^{-1}). Strain NRRL-1195 yielded higher final ethanol as compared to the strain NRRL-1109 when lactose solution was used. As shown in Figure 5.2 b ethanol yields with other media were considerably lower than those obtained with CWP. The yield coefficients of the strains were nearly the same fr CWP (NRRL 1109 =0.52, NRRL-1195= 0.53 g EtOH g^{-1} sugar). The yield coefficients with CW for NRRL-1109 and NRRL-1195 were 0.36 and 0.32 g EtOH g^{-1} $sugar^{-1}$, respectively. The lowest yields were obtained with lactose and NRRL-1195 was better than NRRL-1109. Sugar utilization rates were low for CW and CWP. High sugar utilization rates (590 mg S l^{-1} h^{-1}) were obtained with lactose as depicted in Figure 5.2 c. Ethanol formation rate was maximum (0.25 ml EtOH $l^{-1}h^{-1}$) with CWP solution as shown in Figure 5.2 d. Ethanol formation rates obtained with CW and lactose were 0.15 ml l^{-1} h^{-1} for NRRL-1195 in both media. Based on final ethanol yield, CWP was found to be the most suitable substrate and the *K. marxianus* strain NRRL-1195 the most suitable strain.

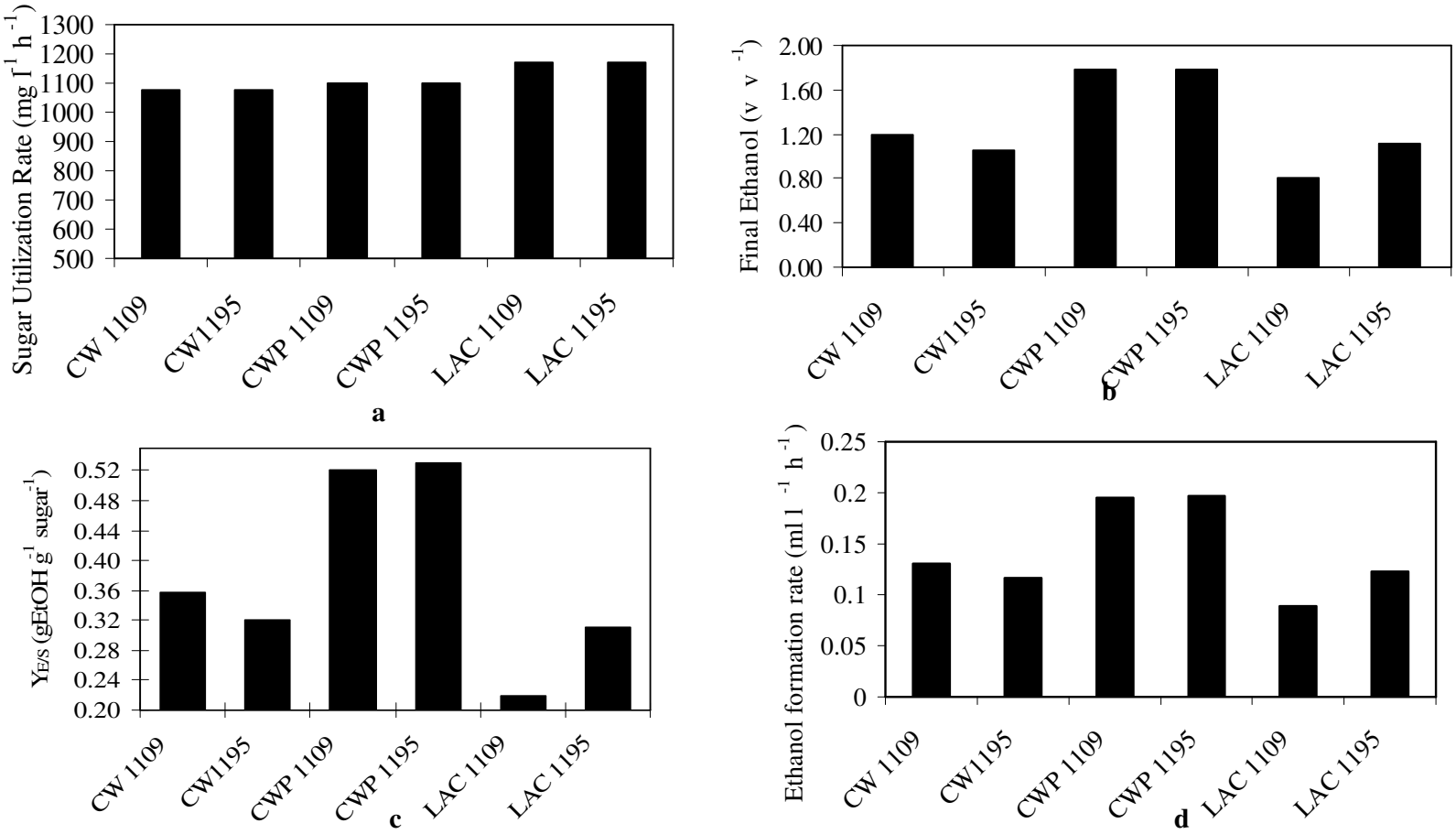


Figure 5.2 **a.** Variation of final ethanol with different strains and media **b.** Variation of yield coefficient with different strains and media **c.** Variation of sugar utilization rate with different strains and media **d.** Variation of overall ethanol formation rate with different strains and media

5.1.2 Effects of Operating Conditions on Ethanol Fermentation by K.Marxianus NRRL-1195

5.1.2.1 Effects of Initial pH

CWP concentration in variable initial pH experiments was 70 g l^{-1} yielding approximately 35 g l^{-1} initial sugar concentration. Experiments were conducted at five different initial pH's varying between 3 and 7. Figure 5.3a shows variation of total sugar (TS) concentration with time for different initial pH's. Total sugar concentration decreased with time and the fermentation was completed in 48 hours for all experiments. Total sugar consumption was faster for initial pH=6 as compared to the others. Time course of variations of percent ethanol (v v^{-1}) concentrations are depicted in Figure 5.3 b. Ethanol concentration in solution increased with time and reached the maximum level after 48 hours. Final ethanol concentration was maximum (1.28 %) for initial pH of 5. No ethanol formation and sugar utilization was observed in the control flask. Variations of media pH with time are depicted in Figure 5.3 c. pH did not change with time for initial pH of 3 and 4. However, the media pH decreased with time within the first 12 hours and reached a steady level around pH = 4.5 when the initial pH was 5 or 6. pH drop was rather sharp within the first 12 hours when initial pH was 7 which stabilized around pH = 5 after 24 hours. As a result of decreasing pH, ORP of the media increased with time as presented in Figure 5.3 d. ORP values increased from -275 ± 25 mV to -200 mV for all experiments except the one with pH =7 which increased to -150 mV at the end of 72 h. Based on final ethanol yield, initial pH of 5 or 6 can be considered as the most suitable pH levels. However, since the changes in pH and ORP were lower for pH= 5, the initial pH of 5 was considered as the most suitable one.

Initial pH also affected the ethanol yield coefficient ($Y_{P/S}$), the rates of ethanol formation and sugar utilization as well as final ethanol concentration. Figure 5.4 a depicts variation of final ethanol concentration with initial pH. The maximum ethanol yield was obtained at initial pH of 5 (1.28% ethanol, v v^{-1}) followed by that obtained at pH = 6 (1.25%, v v^{-1}). Ethanol yields at other pH levels were considerably lower than those obtained at pH of 5 or 6. Ethanol yield constant ($Y_{E/S}$,

g EtOH. g sugar^{-1}) also varied with initial pH as shown in Figure 5.4 b. Almost all of the yield constants were around 0.30 g EtOH g sugar^{-1} except the one at pH = 6 which was about 0.35 g EtOH. g sugar^{-1}. Ethanol formation rate was maximum (0.180 ml Et. l^{-1}h^{-1}) at pH = 5 and 6 as shown in Figure 5.4 c. Sugar utilization rates depicted in Figure 5.4 d were low for initial pH levels of 6 and 7. High sugar utilization rates (700 mg S l^{-1} h^{-1}) were obtained at pH = 3 to 5. Based on the overall results, the initial pH of 5 was selected as the most suitable pH yielding high ethanol formation and sugar utilization rates with the highest final ethanol concentration.

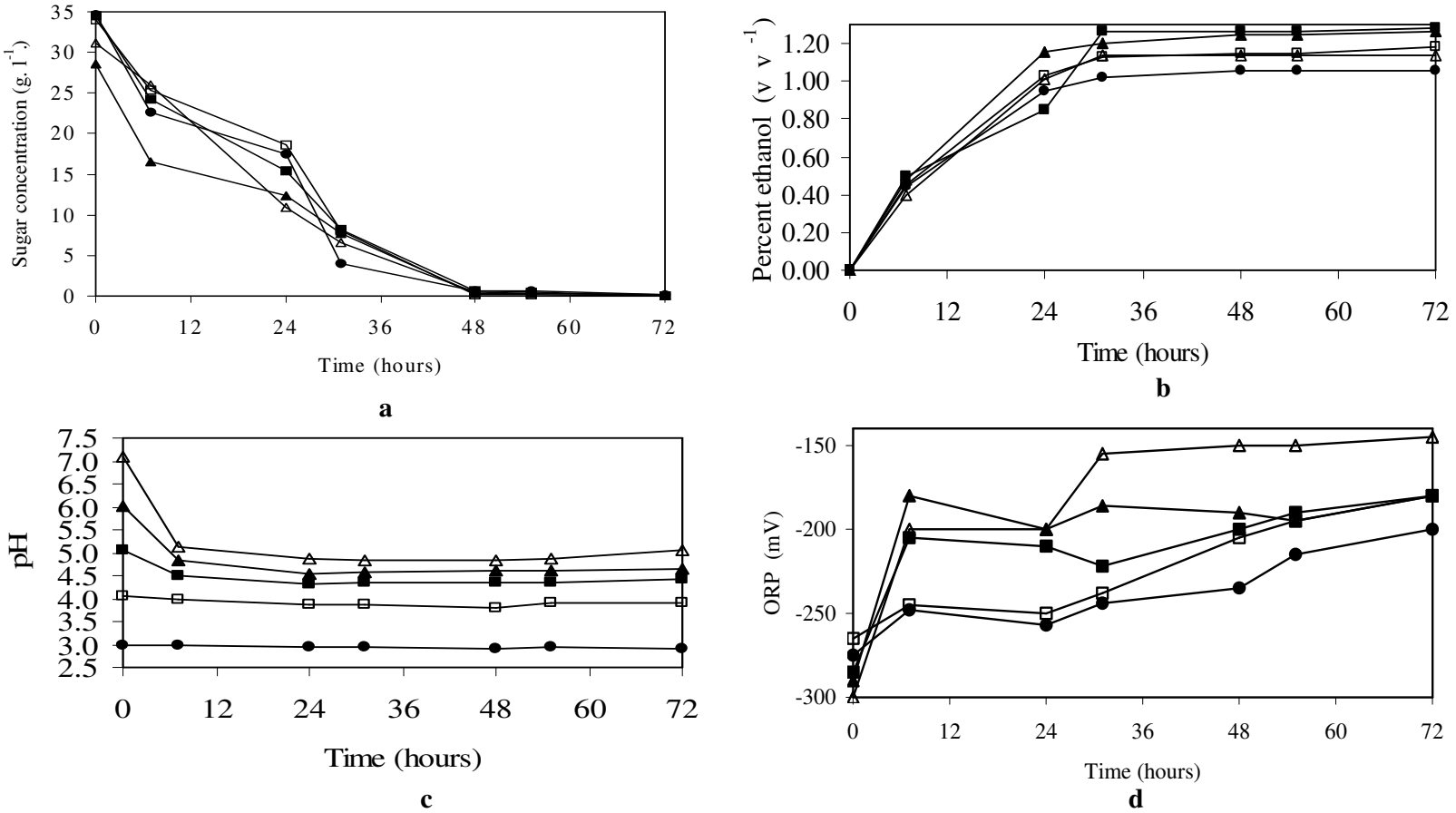


Figure 5.3 **a.** Variation of sugar concentration with time **b.** Variation of percent ethanol with time **c.** Variation of pH with time **d.** Variation of ORP with time. ● pH 3, □ pH 4, ■ pH 5, ▲ pH 6, Δ pH 7

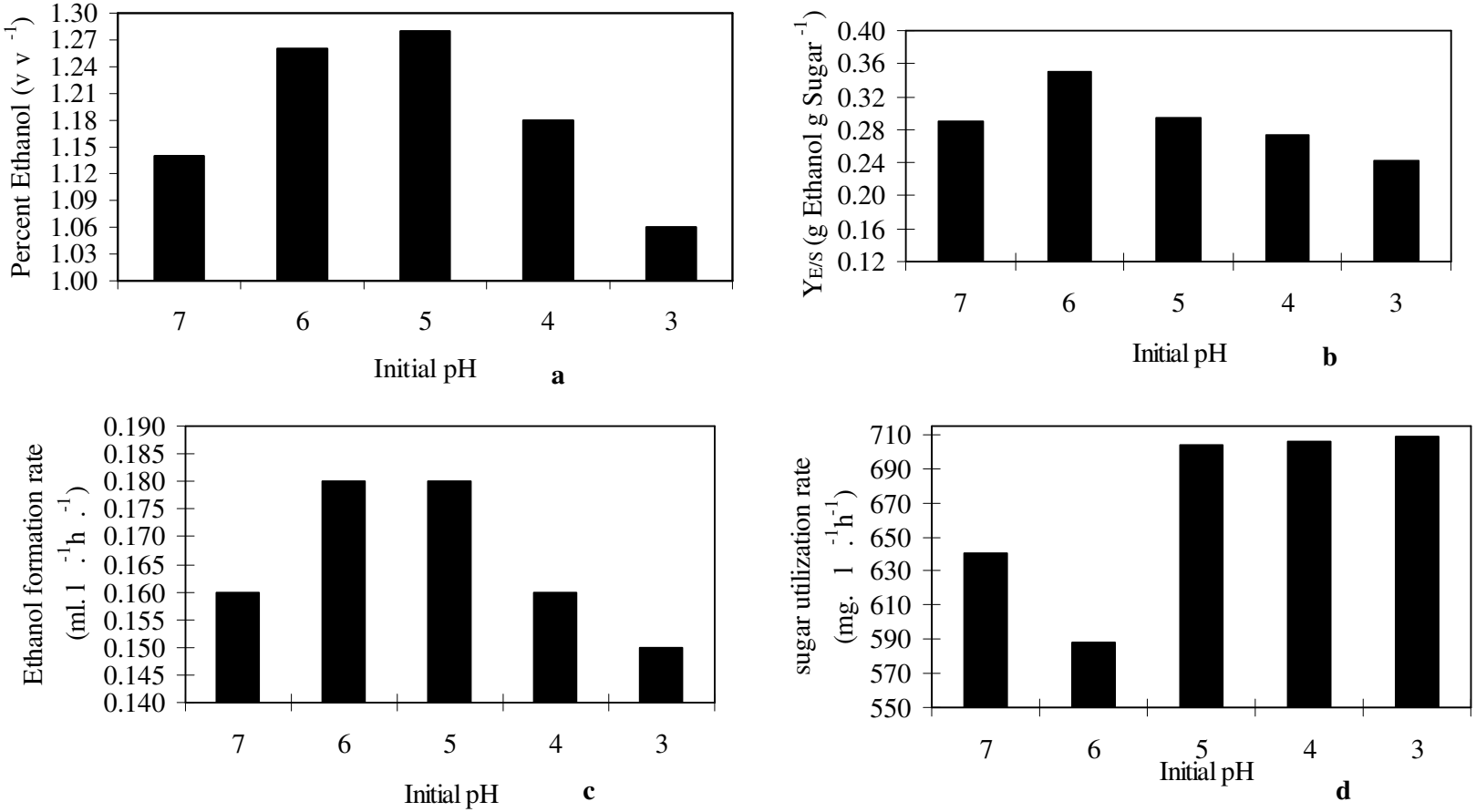
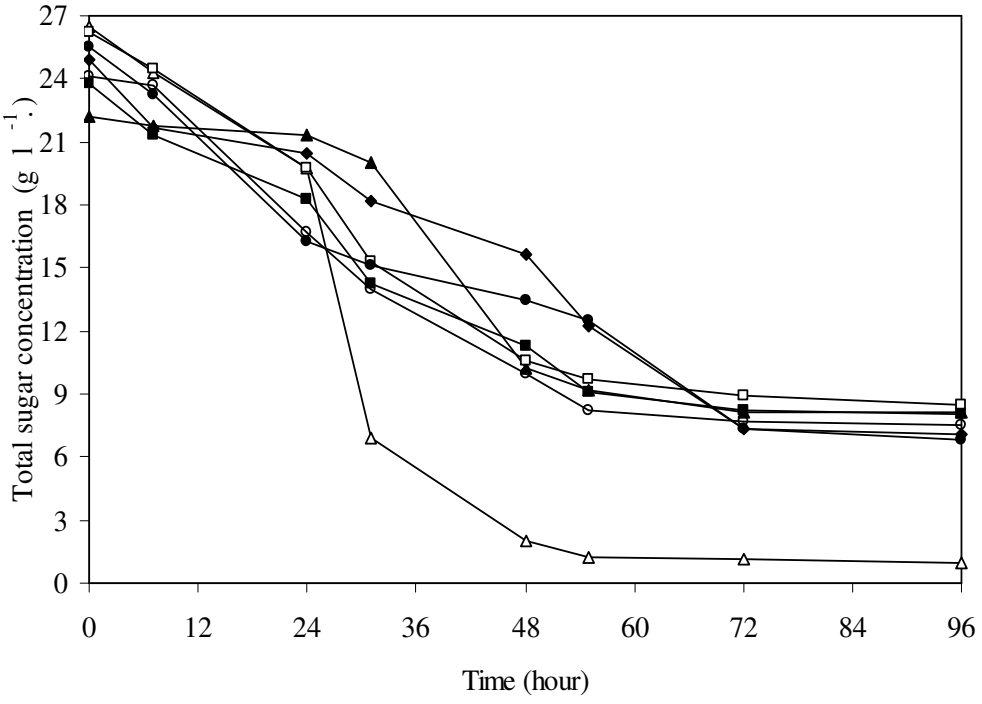


Figure 5.4 **a.** Variation of percent ethanol with initial pH **b.** Variation of yield coefficient with initial pH **c.** Variation of overall ethanol formation rate with inital pH **d.** Variation of sugar utilization rate with initial pH

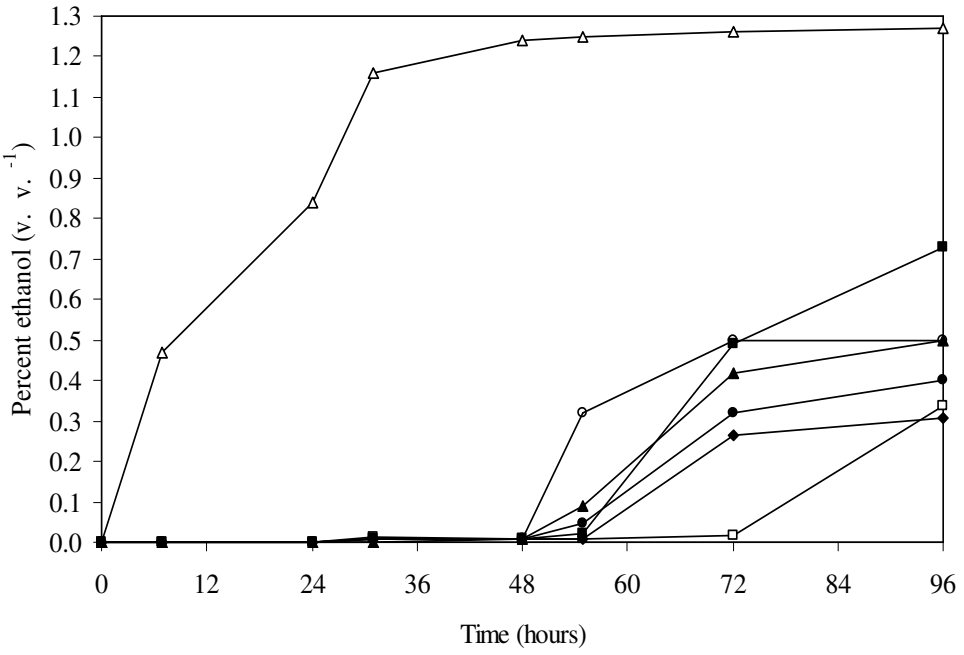
5.1.2.2 Effects of External Nutrient Additions

In order to determine if CWP is nutritionally sufficient for ethanol fermentation, NH_4Cl and KH_2PO_4 salts were added to the 52 g l^{-1} CWP solution (approx. 25 g l^{-1} sugar) and the yields of ethanol formation were experimentally determined. Seven different experiments were performed with different initial N and P contents. In the two experimental flasks the N content of CWP was increased twice and four times by external addition of NH_4Cl while the phosphorous content was constant. In the other two flasks P content of CWP was increased twice and four times while the nitrogen content was constant. The last two flasks contained doubled or quadrupled N and P with external additions. Figure 5.5 a depicts variations of total sugar concentrations with time for 7 experimental flasks containing different amounts of N and P. Fermentation was completed within 72 hours in all flasks. However, the highest sugar utilization was obtained with the CWP solution without any external nutrient addition. Variations of time course of ethanol concentrations for different experimental flasks are shown in Figure 5.5 b. Again the highest final ethanol concentration (1.28%, v v^{-1}) was obtained without any nutrient addition. Ethanol concentrations with external N and P additions varied between 0.70 and 0.30% v v^{-1}. No ethanol formation and sugar utilization was observed in the control flask. Apparently, external N and P additions stimulated cell growth and opressed ethanol formation.

Final ethanol yield , ethanol yield coefficient, the rates of sugar utilization and ethanol formations were also investigated with external N and P additions. Figure 5.6 a depicts final ethanol concentrations for different media compositions. The highest ethanol yield (1.28% v v^{-1}) was obtained with CWP solution without any external N and P sources. Ethanol yields obtained with external N and P sources were considerably lower than that obtained with CWP alone. The ethanol yield coefficient ($Y_{P/S}$) also varied with nutrient additions to the fermentation media as shown in Figure 5.6 b. Again the highest $Y_{P/S}$ (0.39 g E g S^{-1}) was obtained with CWP solution free of any external N and P salts indicating the fact that CWP solution was well balanced in terms of N and P for ethanol fermentation. Sugar utilization and ethanol formation rates are depicted for different media compositions in Figure 5.6 c and



a



b

Figure 5.5 **a.** Variation of sugar concentration with time **b.** Variation of percent ethanol with time. Δ CWP, ▲ CWP+2N+ P, □ CWP+4N+P, ■ CWP+ N+2P, ○ CWP+ N+ 4P, ● CWP+2N+2P, ♦CWP+4N+ 4P

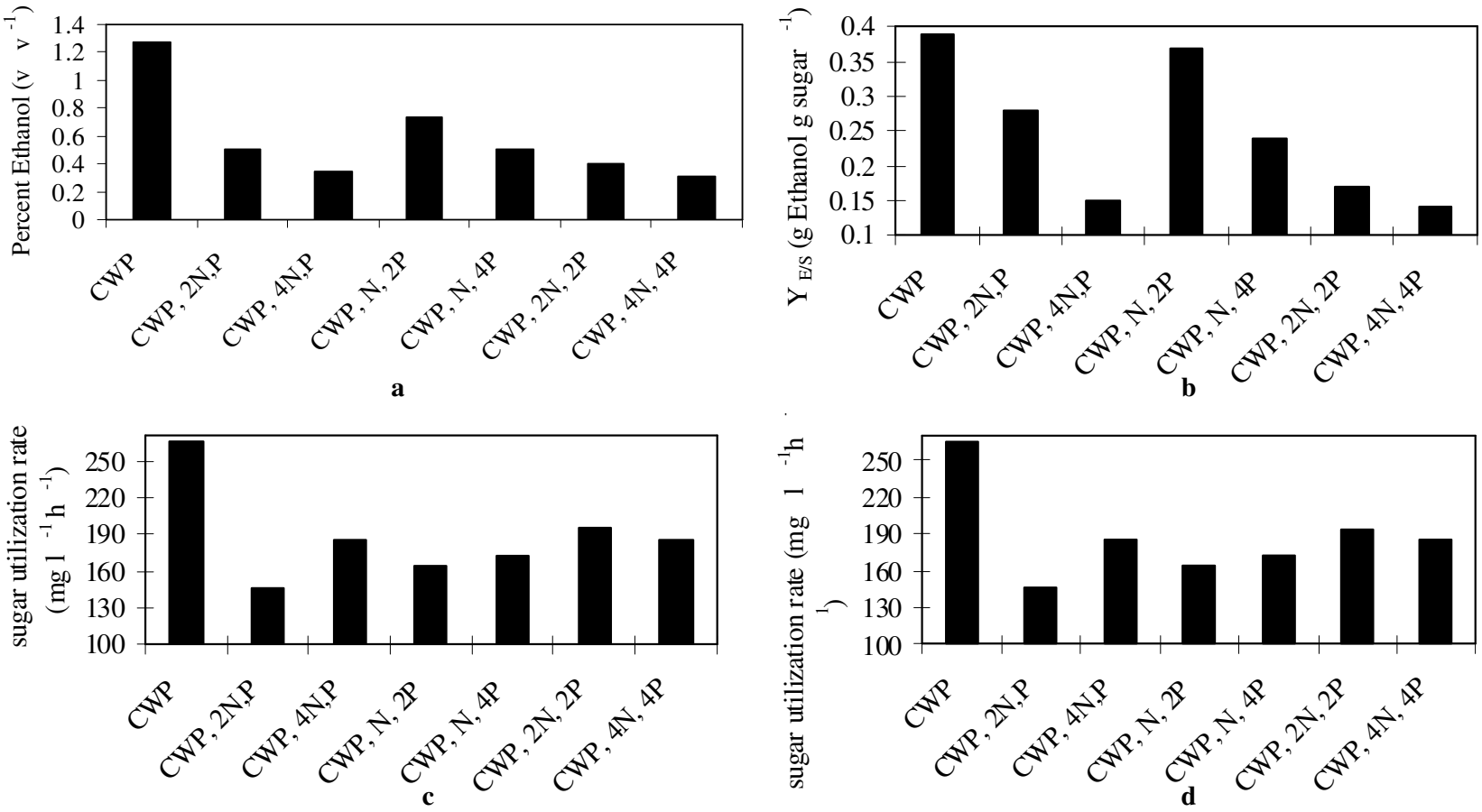


Figure 5.6 **a.** Variation of percent ethanol with initial N, P contents **b.** Variation of yield coefficient with initial N, P contents **c.** Variation of sugar utilization with inital N, P contents **d.** Variation of overall ethanol formation rate rate with initial N, P contents

Figure 5.6 d, respectively. The maximum sugar utilization (270 mg S l^{-1} h^{-1}) and ethanol formation (0.13 ml Et l^{-1} h^{-1}) rates were obtained with the CWP solution without any N and P additions. The results clearly indicated that the N and P contents of CWP were sufficient for ethanol fermentations and any external N and P additions would stimulate cell growth but opress ethanol fermentation.

5.1.2.3 Effects of CWP Concentration on Ethanol Fermentation by K. Marxianus NRRL-1195

Six batch shake flask experiments were carried out with CWP concentration between 52 and 312 g l^{-1} with the corresponding initial sugar concentrations between 26 and 156 g l^{-1}. Figure 5.7 a depicts variation of sugar concentration with time for different CWP concentrations. At low CWP concentrations (52-156 g l^{-1}) sugar utilization was fast resulting in complete sugar utilization within 72 hours. High CWP concentrations above 200 g l^{-1} (sugar concentration above 100 g l^{-1}) caused a lag phase for sugar utilization probably due to high osmotic pressure. Considerable sugar utilization was realized only after 72 hours of incubation at high sugar concentrations above 100 g l^{-1}. Complete sugar utilization was achieved only after 144 hours of incubation at high CWP concentrations above 200 g l^{-1} (sugar > 100 g l^{-1}). Sugar concentration should be kept below 100 g l^{-1} for fast sugar utilization. No sugar utilization was observed in the control flask.

Variations of ethanol concentration with time for different CWP or sugar concentrations are shown in Figure 5.7 b. Ethanol concentration increased with time and reached a constant final concentration at the end of 72 hours of incubation for low CWP concentrations between 52 and 156 g l^{-1} (total sugar = 26-78 g l^{-1}). Similar to sugar utilization, ethanol formation was slow for the first 72 hours for sugar concentrations above 100 g l^{-1} (CWP > 200 g l^{-1}), probably due to osmotic pressure caused by high sugar concentrations. Ethanol formation increased considerably after the first 72 hours of adaptation period for sugar concentrations above 100 g l^{-1}. The maximum final ethanol concentration of 10.5% EtOH (v v^{-1}) was obtained at the end of 216 hours when initial sugar was 156 g l^{-1} (CWP = 312 g l^{-1}). Apparently, high

sugar concentrations above 100 g l^{-1} slowed down ethanol formation; however, improved the final ethanol concentration considerably.

pH of the fermentation media decreased steadily with time and reached a pH level of 4.0 for the CWP concentration of 52 g l^{-1} (total sugar = 26 g l^{-1}). pH values for the other flasks with different CWP concentrations were between 4.1 and 4.3 at the end of 72 hours and dropped to pH= 4.0 at the end of 216 hours as shown in Figure 5.7 c. Oxidation reduction potentials (ORP) varied between -200 mV and -120 mV and reached a steady level of nearly -150 mV at the end of 216 hours of fermentation in all experimental flasks (Figure 5.7 d).

Variations of ethanol yield (%, v v^{-1}), percent sugar utilization, and ethanol yield coefficient with the CWP concentration at the end of 216 h of incubation are depicted in Figure 8. As shown in Figure 5.8 a, the final ethanol concentration increased with the CWP or sugar concentration yielding nearly 10.5% (v v^{-1}) ethanol with 312 g l^{-1} CWP (156 g sugar l^{-1}) while ethanol yield was only 1.7% (v v^{-1}) with 52 g l^{-1} CWP (26 g sugar l^{-1}). Percent sugar utilizations at the end of 216 hours were above 98% for all CWP concentrations except with CWP of 200 g l^{-1} which yielded 96.5% sugar utilization (Figure 5.8 b). Ethanol yield coefficient (Y_{EtOH} , g EtOH g^{-1} sugar) also varied with the CWP concentration resulting in maximum yield coefficient of 0.54 g EtOH g $sugar^{-1}$ with 312 g l^{-1} CWP or 156 g l^{-1} initial sugar concentration. The yield coefficient varied between 0.35 and 0.54 g EtOH g $sugar^{-1}$ depending on the CWP concentration (Figure 5.8 c). Variation of the ratio of experimental and theoretical yield coefficients with the CWP concentration is depicted in Figure 5.8 d. The theoretical ethanol yield from lactose fermentation is $Y_{E/S}$ = 0.54 g EtOH g^{-1} lactose. The Y_E / Y_T ratio varied between 0.6 and 1.0 with the maximum value obtained at 312 g l^{-1} CWP concentration.

The overall rate of sugar utilization and ethanol formation also increased with increasing initial CWP or sugar concentration as shown in Figure 5.9. When CWP concentration increased from 52 to 312 g l^{-1} (sugar from 26 to 156 g l^{-1}), the overall rate of sugar utilization increased from 110 to 670 mg sugar l^{-1} h^{-1} almost linearly indicating possible substrate limitation (Figure 5.9 a). Similarly, the overall rate of ethanol formation increased from 0.07 to 0.49 ml EtOH l^{-1} h^{-1} when CWP

concentration increased from 52 to 312 g l^{-1} (sugar from 26 to 156 g l^{-1}) (Figure 5.9 b). The fact that the maximum ethanol formation and sugar utilization rates were obtained with the highest sugar concentration indicated no substrate or product inhibitions, but only substrate (sugar) limitations within the experimental range of CWP (52-312 g l^{-1}).

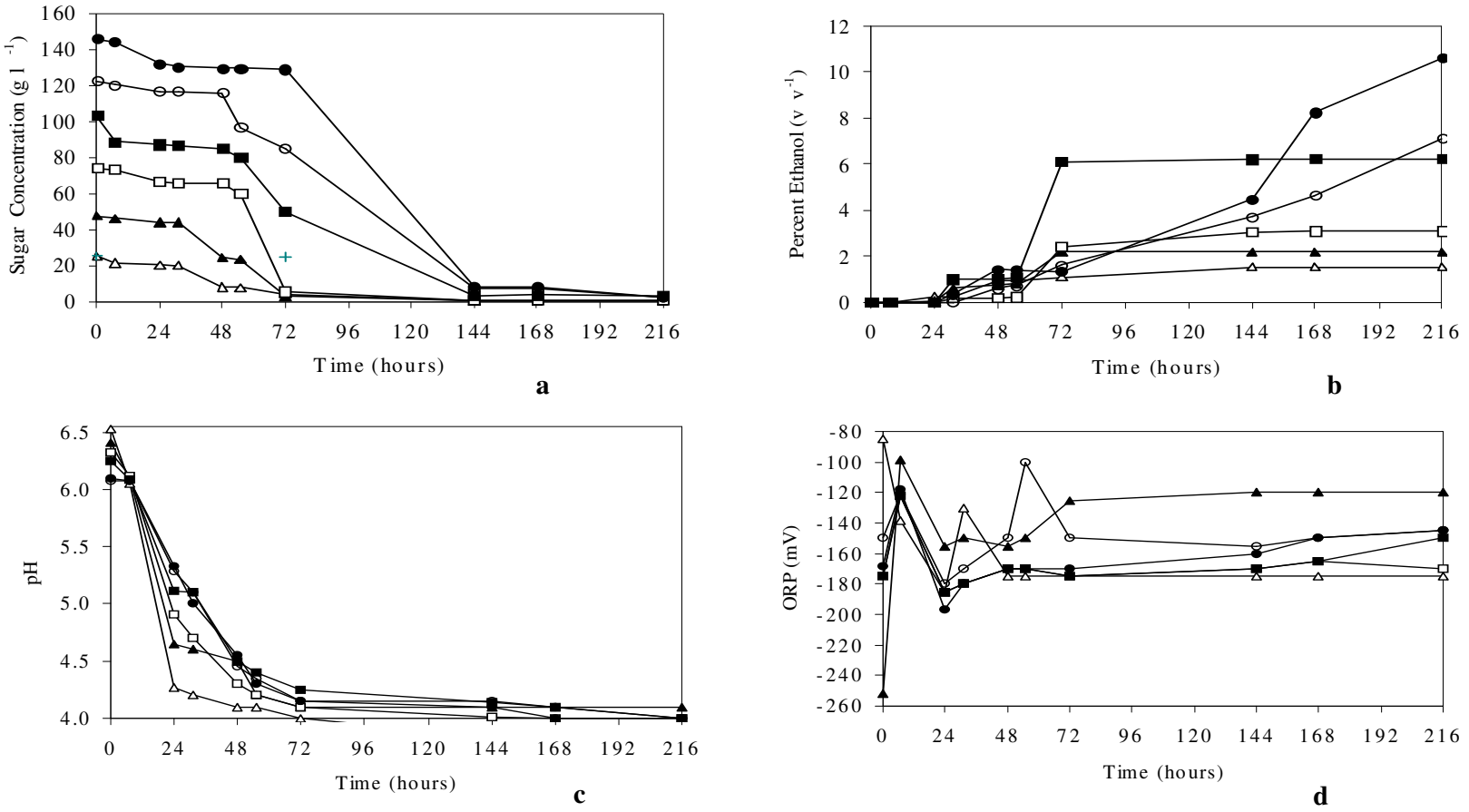


Figure 5.7 **a.** Variation of sugar concentration with time **b.** Variation of percent ethanol with time **c.** Variation of pH with time **d.** Variation of ORP with time. CWP concentrations (g l^{-1}) Δ 52, ▲ 104, □ 156, ■ 208, ○260, ● 312

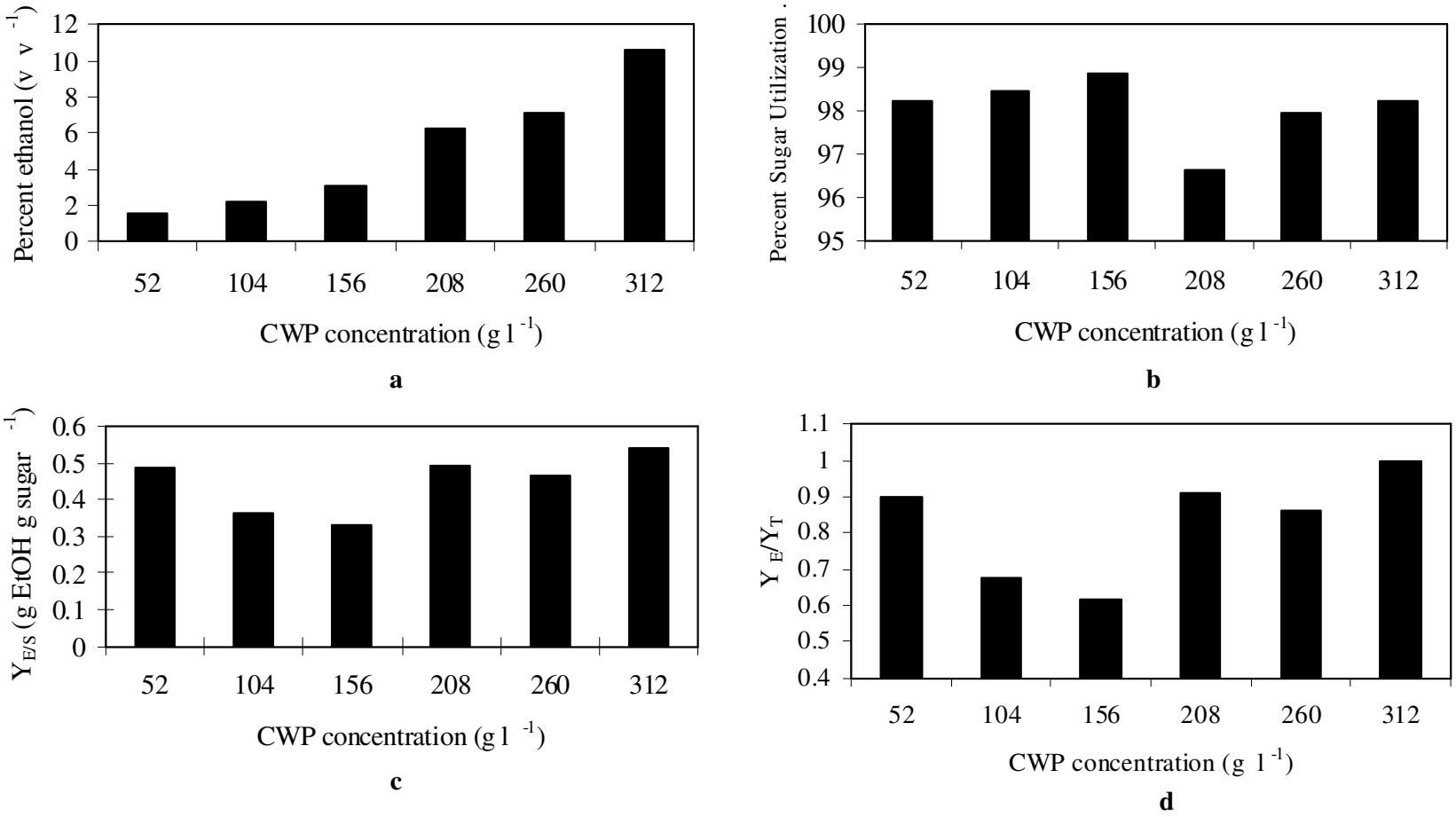
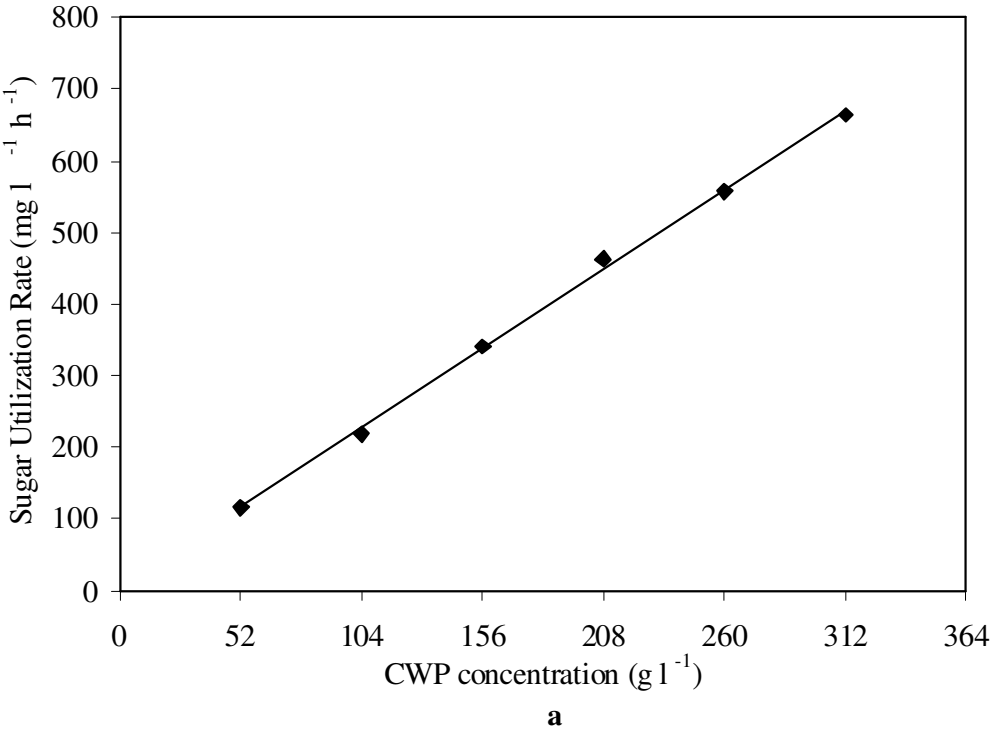
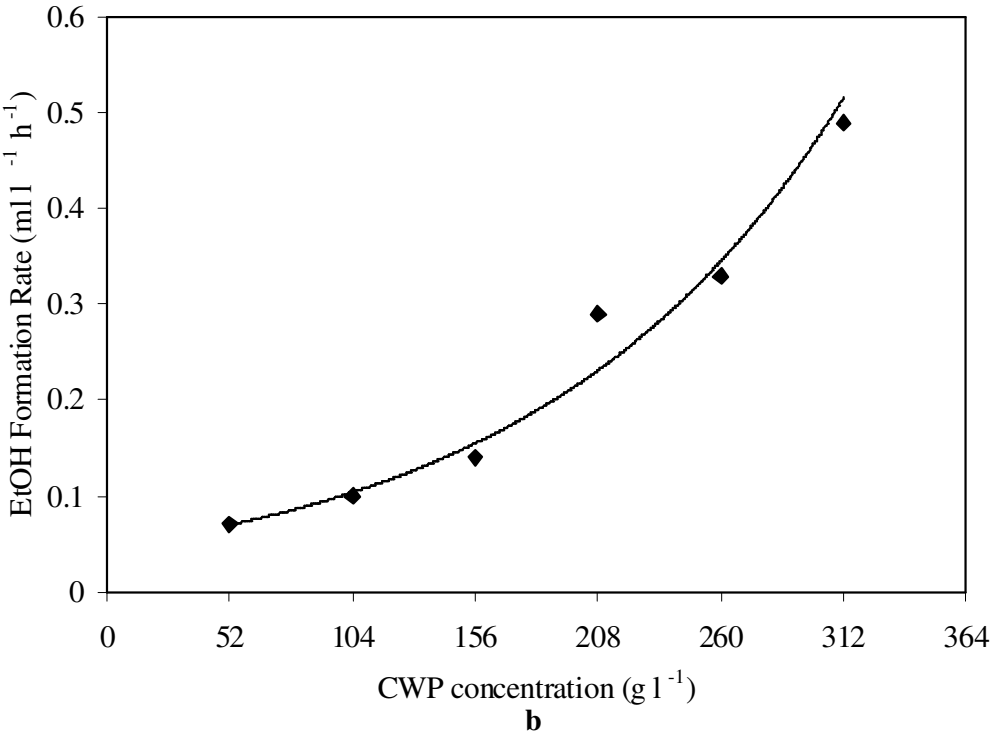


Figure 5.8 **a.** Variation of percent ethanol with the initial CWP concentration **b.** Variation of percent sugar utilization with CWP concentration **c.** Variation of yield coefficient with the initial CWP concentration **d.** Variation of Y_E/Y_T with the initial CWP concentration



a



b

Figure 5.9 **a.** Variation of overall sugar utilization rate with CWP concentratation **b.** Variation of overall rate of ethanol formation with CWP

5.1.3 Comparison of Ethanol Fermentation of CWP by Two Different Kluyveromyces Marxianus Strains

Performance of *Kluyveromyces marxianus* DSMZ 7239 and NRRL 1195 strainswere compared for ethanol formation from CWP solution were compared in batch experiments. Experiments were performed at pH 5 and ORP was set to -250 with 200 mg l^{-1} Na- thioglycolate. The total incubation time was 96 hours. Figure 5.10 depicts comparison of the performances of the two *K. marxianus* strains. Figure 5.10a shows variation of total sugar (TS) concentration with time. Total sugar concentration decreased with time. Total sugar consumption was slower for NRRL-1195. Time course of variations of percent ethanol (v v^{-1}) concentrations are depicted in Figure 5.10b. Ethanol concentration in solution increased with time and reached the maximum level after 42 hours (3.5%) with DSMZ 7239. Variations of media pH with time are depicted in . Figure 5.10c. The pH decreased with time and reached to 4 with NRRL 1195 and nearly 4.4 with DSMZ 7239. ORP of the media decreased with time as presented in Figure 5.10d. For NRRL 1195 and DSMZ 7239, ORP values decreased from -100 ± 25 mV to approximately -175 mV and -275 mV respectively.

Figure 5.11a depicts variation of ethanol yield coefficients for different strains. The maximum ethanol yield was obtained with DSMZ 7239 and was closer to the theoretical ethanol yield coefficient (0.54 g EtOH/ g sugar). As shown in Figure 5.11b, the maximum ethanol concentration for the DSMZ 7239 was higher than NRRL 1195. The maximum ethanol concentrations for the strains were 3.1 % for NRRL 1195 and 3.35 % DSMZ 7239. The initial yeast concentration in the flasks was 4.6 g l^{-1}.High specific sugar utilization rates (2540 mg S l^{-1} h^{-1}) were obtained with DSMZ 7239 as depicted in Figure 5.11c. Specific ethanol formation rate was high (4 ml EtOH $g^{-1}h^{-1}$) with the DSMZ 7239 as shown in Figure 5.11d. On the basis of final ethanol yield, the yeast strain DSMZ 7239 was found to be the most suitable strain and was used in further experiments.

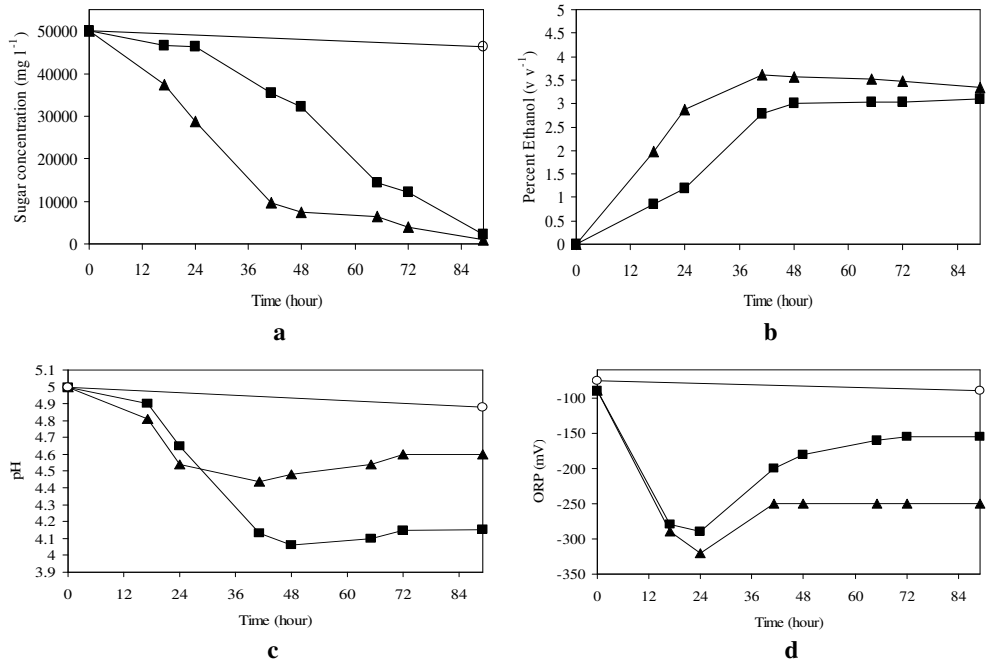


Figure 5.10 **a.** Variation of sugar concentration with time, **b.** Variation of percent ethanol concentration with time, **c.** Variation of pH with time 1d. Variation of ORP with time ■NRRL-1195 ▲ DSMZ 7239 , ○ Control

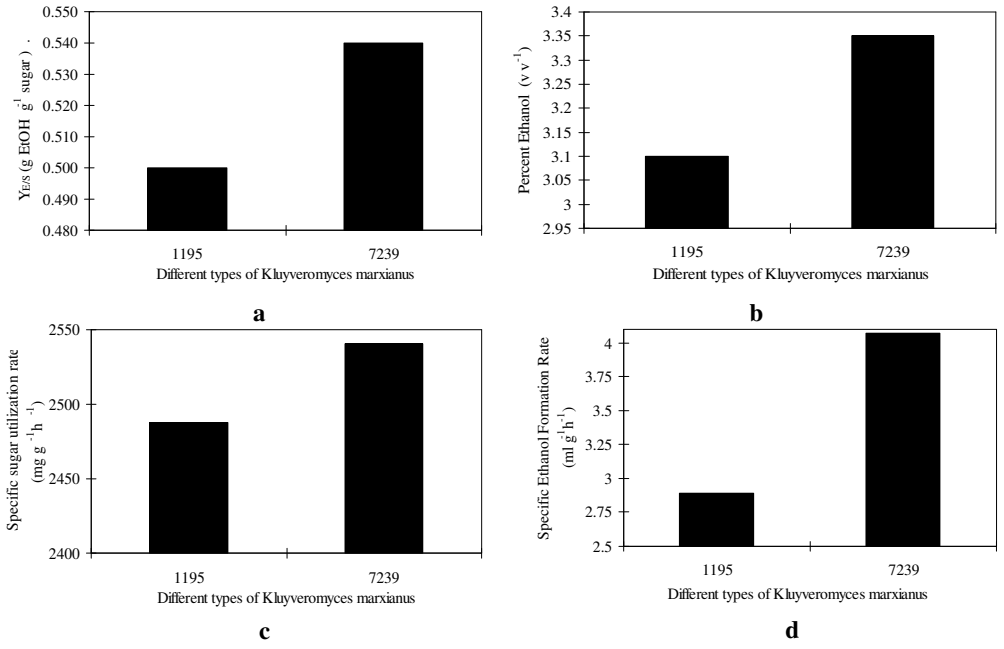


Figure 5.11 **a.** Ethanol yield coefficient for the different strains **b.** Final ethanol concentrations for the different strains **c.** Specific sugar utilization rates for the different strains **d. S**pecific ethanol formation rates for the different strains

5.1.4 Effects of Environmental Conditions on Ethanol Fermentation of CWP by K. Marxianus DSMZ-7239

5.1.4.1 Effects of Initial pH

Variable pH experiments were carried out with *Kluyveromyces marxianus* DSMZ 7239. Five different flasks were prepared to find out the most suitable pH for ethanol formation from CWP solution. Experiments were conducted at pH 3, 4, 5, 6 and 7. Figure 5.12 a shows variation of sugar concentration with time at different initial pH levels. Sugar utilization was almost complete within 24 h in all flasks except the one at initial pH of 5.0. Sugar content of the medium reached the minimum level in 55 hours. Time course of variations of percent ethanol (v v^{-1}) concentrations are depicted in Figure 5.12 b. Ethanol concentrations increased with time and reached the maximum level after 48 hours. Final ethanol concentration was maximum (3.43 %) for the initial pH of 5. Variations of pH with time are depicted in Figure 5.12 c. pH did not change with time for initial pH of 3 and 4. However, the media pH decreased with time within the first 24 hours and reached a steady level around pH = 4.5 when the initial pH was 5 or 6. As a result of decreasing pH, ORP of the media was also changed with time as presented in Figure 5.12 d. ORP values increased from -220 ± 25 mV to -180± 25 mV for pH 3 and 4. On the basis of final ethanol yield, initial pH of 5 or 6 can be considered as the most suitable pH levels. However, since the changes in pH and ORP were lower for pH 5, the initial pH of 5 was considered as the most suitable one.

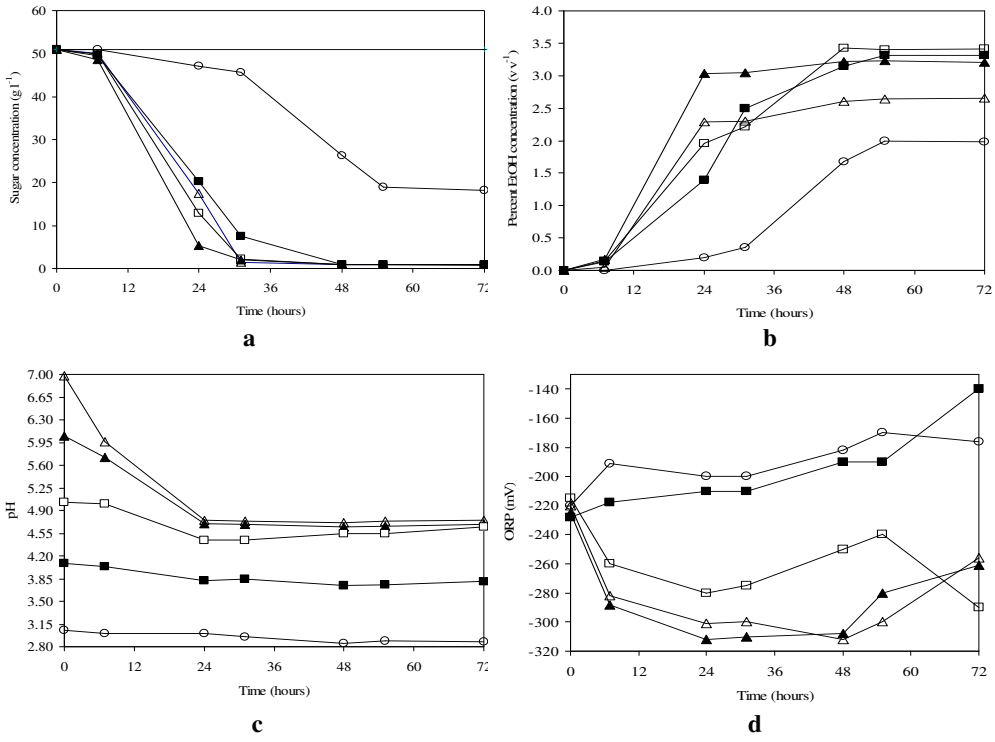


Figure 5.12 **a.** Variation of sugar concentration with time, **b.** Variation of percent ethanol concentration with time, **c.** Variation of pH with time **d.** Variation of ORP with time, pH : Δ7, ▲6, □5, ■4, o3

Initial pH also affected the ethanol yield coefficient ($Y_{E/S}$), the rates of ethanol formation and sugar utilization as well as the final ethanol concentration. Ethanol yields at other pH levels were considerably lower than those obtained at pH of 5 or 6. Ethanol yield constant ($Y_{E/S}$, g EtOH. g sugar^{-1}) also varied with initial pH as shown in Figure 5.13 a. The maximum ethanol yield constant was obtained at pH 5. Figure 5.13 b depicts variation of final ethanol concentration with the initial pH. The maximum ethanol concentration was obtained at initial pH of 7 (4.75%, v v^{-1}) followed by that obtained at pH = 6 and 5 (4.68% and 4.64% v v^{-1}) respectively. Sugar utilization rates were nearly the same (≈1050 mg S. l^{-1}h^{-1}) at pH = 7,6 and 5 as shown in Figure 5.13 c. The highest ethanol formation rate was obtained at pH 5 (0.71 ml EtOH. l^{-1}h^{-1}) On the basis of overall results the initial pH of 5 was selected as the most suitable pH yielding high ethanol formation and sugar utilization rates with the highest final ethanol concentration.

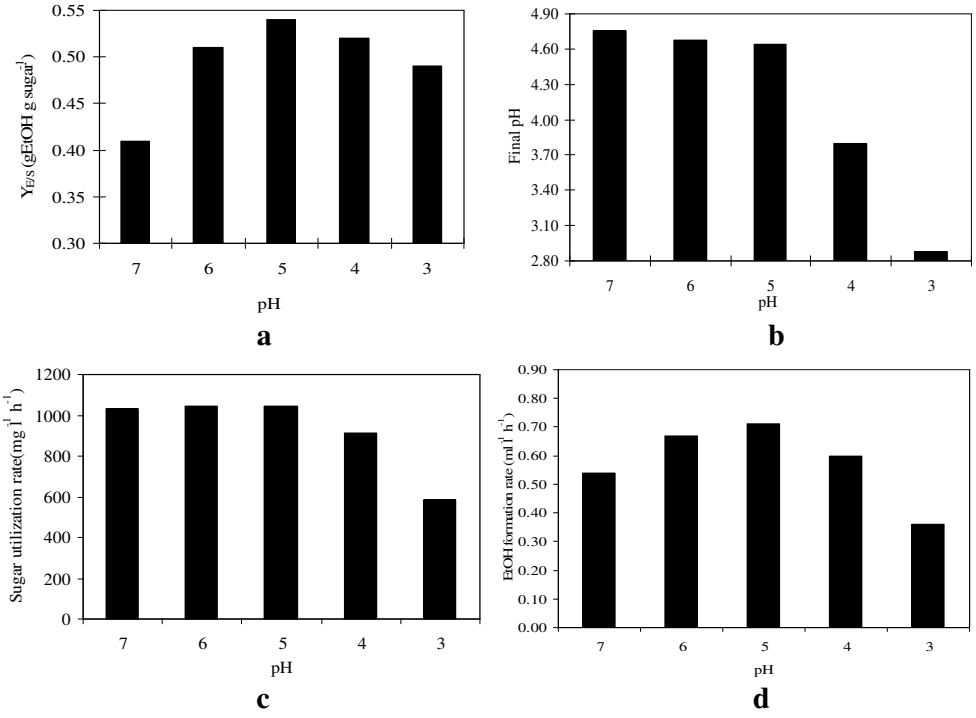


Figure 5.13 **a.** Variation of percent ethanol with initial pH **b.** Variation of yield coefficient with initial pH **c.** Variation of sugar utilization rate with initial pH **d.** Variation of overall ethanol formation rate with initial pH

5.1.4.2 Effects of Initial ORP

Five different flasks were prepared to determine the most suitable initial ORP value for ethanol formation from CWP solution. The initial ORP was adjusted with the addition of different amounts of Na-thioglycolate to the experimental flasks. 50, 100, 200, 250 and 300 mg l^{-1} Na-thioglycolate concentrations were added to obtain -20, -80, -140, -158, -163 mV ORP's respectively. Figure 5.14 a shows time course of variation of sugar concentration at different initial ORP levels. Sugar utilization was almost complete in 24 h for all ORP levels. Sugar concentration in the flasks containing 50, 100, 250 mg l^{-1} Na-thioglycolate decreased to nearly 12 g l^{-1} while the final sugar in the flasks containing 200, 300 mg l^{-1} Na-thioglycolate was nearly 4.5 g l^{-1} sugar at the end of 72 hours. Time course of variations of percent ethanol (v v^{-1}) concentrations are depicted in Figure 5.14b. Ethanol concentration increased with time at all ORP levels. Final ethanol concentration was maximum (3.63 %) for the initial Na-thioglycolate concentration of 200 mg l^{-1} in 55 hours. Variations of pH

with time are depicted in Figure 5.14c. pH decreased in all flasks to 4.5, and then increased to 4.95 at 55 hours. This pH increase may be because of the ethanol formation. Figure 5.14 d depicts variation of ORP with time. ORP decreased with time yielding final ORP's of -85, -170, -250, -280, -295 mV in the flasks containing 50, 100, 200, 250, 300 mg l^{-1} Na-thioglycolate, respectively. On the basis of final ethanol concentration, initial Na-thioglycolate concentration 200 mg l^{-1} can be considered as the most suitable Na-thioglycolate concentration.

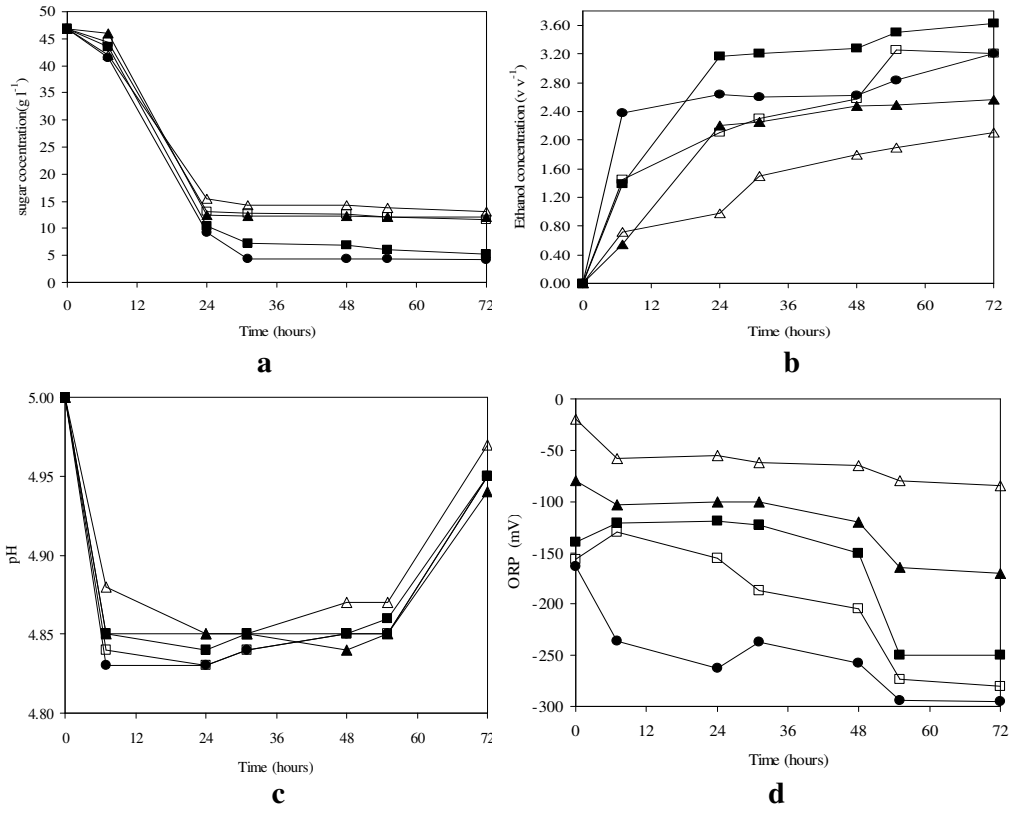


Figure 5.14 **a.** Variation of sugar concentration with time, **5b.** Variation of percent ethanol concentration with time, **5c.** Variation of pH with time **5d.** Variation of ORP with time Na-thioglycolate (mgl^{-1}): Δ 50 , ▲100 , □ 200 , ■ 250 , o 300

Initial Na-thioglycolate also affected the ethanol yield coefficient ($Y_{P/S}$), the rates of ethanol formation and sugar utilization as well as final ethanol concentration. Ethanol yield constants were the same as the theoretical yield coefficient (0.54 g EtOH g S^{-1}) for 200, 250, 300 mg l^{-1} Na-thioglycolate concentration as shown in

Figure 5.15 a. Figure 5.15 b depicts final ethanol concentrations at different ORP levels. The maximum ethanol concentration was obtained with the flask containing 200 mg l^{-1} Na-thioglycolate (3.63 %). Figure 5.15 c depicts sugar utilization rate for different Na-thioglycolate concentrations. The flasks containing 50 and 100 mg l^{-1} Na-thioglycolate concentrations resulted in the maximum sugar utilization rates of 470 and 478 mg l^{-1} h^{-1}, respectively. Figure 5.15d depicts ethanol formation rates for different Na-thioglycolate concentrations. The maximum ethanol formation rate (0.65 ml $l^{-1}h^{-1}$) was obtained with the flask containing 200 mg l^{-1} Na-thioglycolate. On the basis of final ethanol, yield coefficient and ethanol formation rate, the initial Na-thioglycolate concentration 200 mg l^{-1} was chosen as the most suitable with an initial ORP of -140 mV.

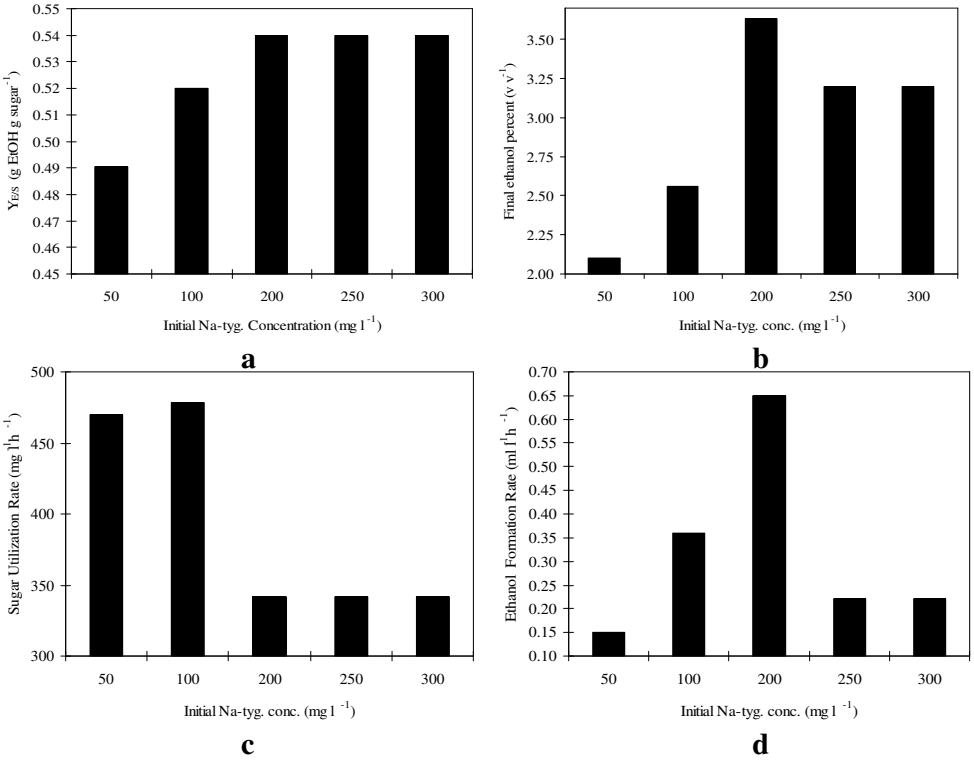


Figure 5.15 **a.** Variation of percent ethanol with initial Na-thioglycolate **b.** Variation of yield coefficient with initial Na-thioglycolate **c.** Variation of sugar utilization rate with initial Na-thioglycolate **d.** Variation of overall ethanol formation rate with initial Na-thioglycolate

5.1.5 Experiments with different CWP and yeast concentrations using K. marxianus DSMZ-7239

5.1.5.1 Effect of Substrate (CWP) Concentration

The cheese whey powder (CWP) concentration varied between 52 and 312 g l^{-1} with total soluble sugar (TS) contents between 26 and 156 g l^{-1} in this set of batch experiments while the initial biomass concentration was constant at 0.5 g l^{-1}. Variations of total soluble sugar and ethanol concentrations with time are depicted in Figure 5.16 a and b, respectively for different initial CWP concentrations. Sugar utilization was almost completed within 72 hours when CWP concentration was less than 156 g l^{-1} (TS< 78 g l^{-1}). Complete sugar utilization took longer time when CWP was larger than 156 g l^{-1} (Figure 5.16 a) due to substrate inhibition at high sugar concentrations. Ethanol formation also reached the maximum level after 72 hours of incubation when CWP was less than 156 g l^{-1} (TS < 78 g l^{-1}) while complete ethanol formation took longer for higher CWP concentrations. An incubation time of 72 hours was considered in all further calculations. The pH values dropped from an initial level of 5 to 4.5 at the end of 72 hours when CWP was less than 156 g l^{-1}. The final pH for CWP concentrations above 156 g l^{-1} was between 4.7 and 4.9 at the end of 72 hours. The ORP decreeased from -150 mV to nearly -350 mV in all experiments, except the one with 52 g l^{-1} CWP for which the final ORP was -250 mV at the end of 72 hours. Increase in biomass concentration was less than 10% in all flasks. There was no ethanol formation or sugar utilization in the control flask.

Variations of the ethanol yield coefficient and final ethanol concentration (72 hours) with the initial CWP concentration are depicted in Figure 5.17 a and b. The ethanol yield coefficient ($Y_{P/S}$) was almost constant at the theoretical value (0.54 g EtOH. g $lactose^{-1}$) for CWP concentrations below 156 g l^{-1} which dropped sharply at high CWP levels because of inhibitory effects of high sugar concentrations (Figure 5.17 a). Final ethanol concentrations also increased with the initial sugar or CWP concentration up to CWP of 156 g l^{-1} and then decreased with increasing CWP concentrations above 156 g l^{-1} due to substrate inhibition (Figure 5.17 b). The maximum ethanol concentration of 5.2% (v v^{-1}) was obtained with 156 gl^{-1} CWP concentration, which is almost equal to the theoretical yield.

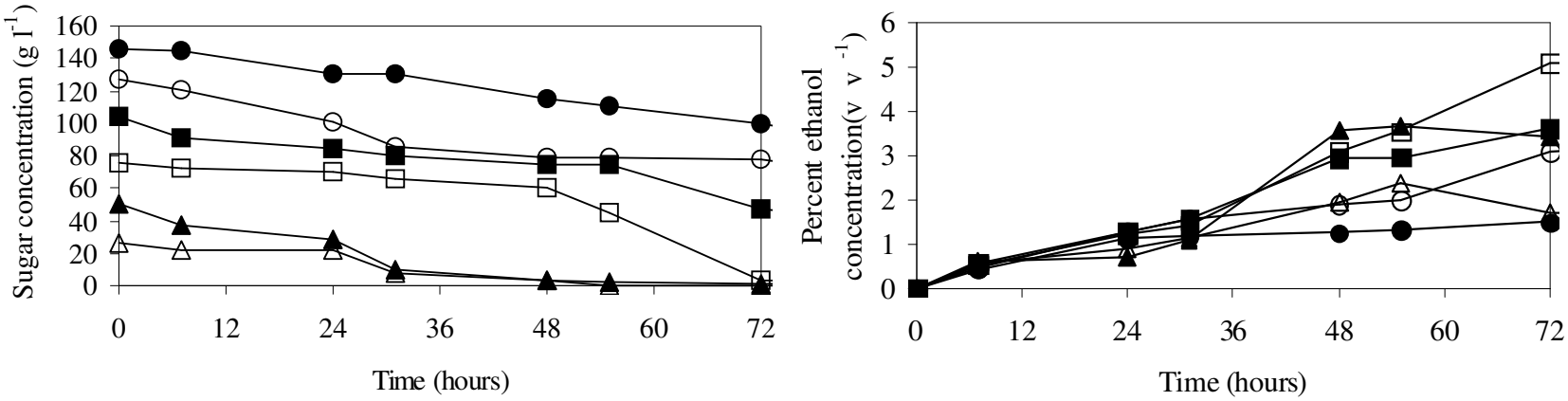


Figure 5.16 **a.** Variation of sugar concentration with time, **b.** Variation of percent ethanol concentration with time. Cheese whey powder (CWP) concentration (g l^{-1}): Δ 52, ▲ 104, □ 156, ■ 208, ○ 260, ● 312

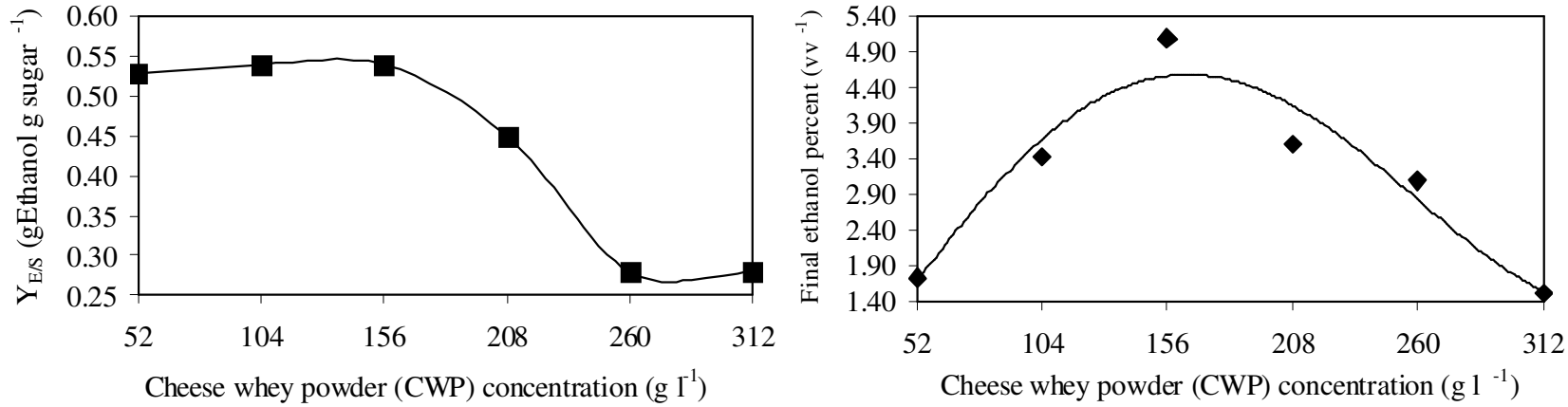


Figure 5.17 **a.** Variation of yield coefficient with CWP concentrations, **b.** Variation of percent final ethanol with CWP concentrations

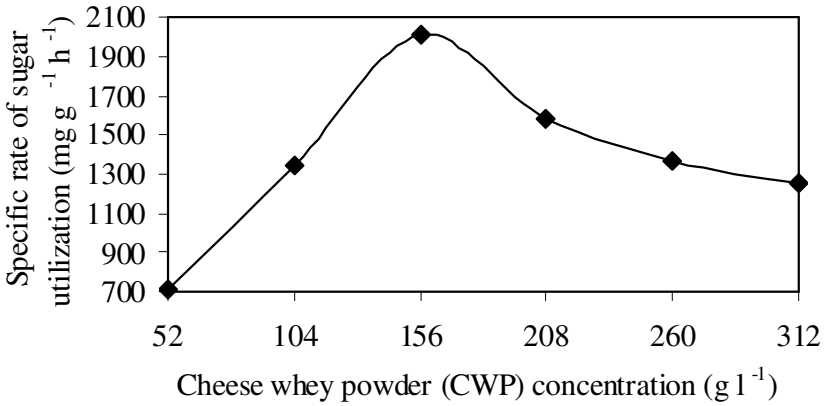
Variations of specific rates of sugar utilization and ethanol formation with CWP concentration are shown in Figure 5.18 a and b. Specific rates (R = (So- S)/(t.X), g sugar/g biomass.h) were calculated for the first 72 hours. The specific rate of sugar utilization increased with sugar or CWP concentrations up to 156 g l^{-1} CWP (Total sugar = 78 g l^{-1}) indicating substrate limitations at low sugar concentrations. However, the rate decreased with increasing sugar concentrations above 78 g l^{-1} (CWP> 156 g l^{-1}) due to substrate inhibition at high sugar concentrations (Figure 5.18 a). Similar trends were also observed in the specific rate of ethanol formation ((P-Po)/ (t X), g EtOH/ g biomass.h). Ethanol formation rate for the first 72 hours increased with sugar concentration at low CWP concentrations below 156 g l^{-1} (TS< 78 g l^{-1}) due to substrate limitations. However, ethanol formation rate steadily decreased with increasing CWP concentrations for CWP larger than 156 g l^{-1} (TS > 78 g l^{-1}) due to substrate inhibition as a result of high osmotic pressure at high sugar concentrations (Figure 5.18 b). Sugar concentration should not exceed 78 g l^{-1} (CWP< 156 g l^{-1}) for high rate and extent of ethanol formation.

5.1.5.2 Effect of Initial Yeast Concentration

Biomass (yeast) concentration is another important parameter affecting the rate and extent of ethanol formation from CWP. A series of batch shake flask experiments were performed with varying initial biomass concentrations between 170 and 1020 mg l^{-1} with a constant CWP concentration of 100 g l^{-1}. The results are depicted in Figure 5.19 and Figure 5.20. Figure 5.19 a and b depict variations of total soluble sugar and ethanol concentrations with time for different initial biomass concentrations. Sugar utilization was completed within 24 and 30 hours when biomass concentrations were above 850 mg l^{-1} and 510 mg l^{-1}, respectively. However, sugar utilization was rather slow for biomass concentrations below 510 mg l^{-1} since the rate is directly proportional with the biomass concentration. Sugar utilization was completed after 72 hours of fermentation when biomass concentration was less than 510 mg l^{-1} (Figure 5.19 a). Ethanol formation also reached the maximum level after 72 hours of incubation when biomass concentration was above 510 mgl^{-1}. Nearly 120 hours of fermentation times were required for maximum ethanol formation when biomass concentrations were lower than 510 g l^{-1} as shown

in Figure 5.19 b. pH values in experimental flasks decreased from an initial pH of 5 to pH 4.6- 4.8 depending on the initial biomass concentrations. Therefore, pH variations were not significant to require pH control. The final oxidation reduction potentials (ORP) at the end of 72 hours were between -250 and -275 mV with an initial ORP of -250 mV for all experimental flasks. There was no sugar utilization and ethanol formation in the control flask free of biomass.Figure 5.20 a and b depict variations of volumetric rates of sugar utilization and ethanol formation with the initial yeast concentration. The time period considered for calculating the rates were until complete utilization for sugar (24, 31 and 48 hours for different biomass concentrations) and 120 hours for ethanol, since ethanol formation continued after complete sugar consumption. The volumetric rate of sugar utilization increased with biomass concentration almost linearly yielding nearly 2200 mg l^{-1} h^{-1} sugar utilization rate at 1020 mg l^{-1} biomass concentration (Figure 5.20 a). Ethanol formation rate also increased with biomass concentration as shown in Figure 5.20 b. The maximum ethanol formation rate of 0.305 ml l^{-1} h^{-1} was obtained with 1020 mg l^{-1} initial biomass concentration.

There are no literature studies on ethanol fermentation of cheese whey powder solution. As compared with the literature studies on cheese whey fermentations (Domingues et al., 2001; Kourkoutas et al., 2002 a,b; Silveira et al., 2005; Grba et al., 2002; Zafar and Owais, 2006), higher ethanol yields and rates were obtained in our study especially at high biomasss concentration of 1000 mg l^{-1} and sugar concentration of 78 g l^{-1}.



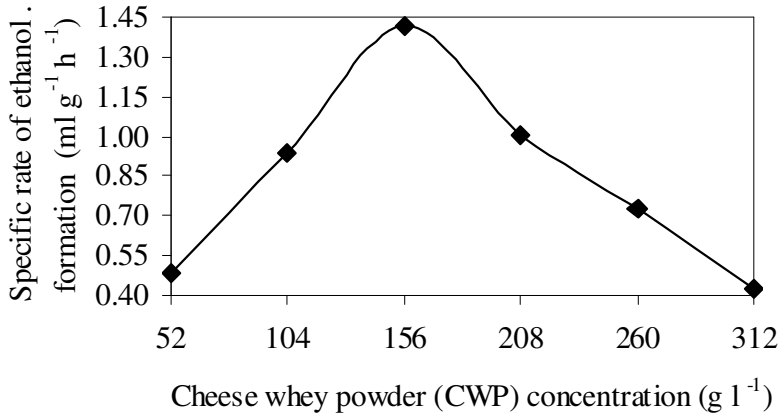
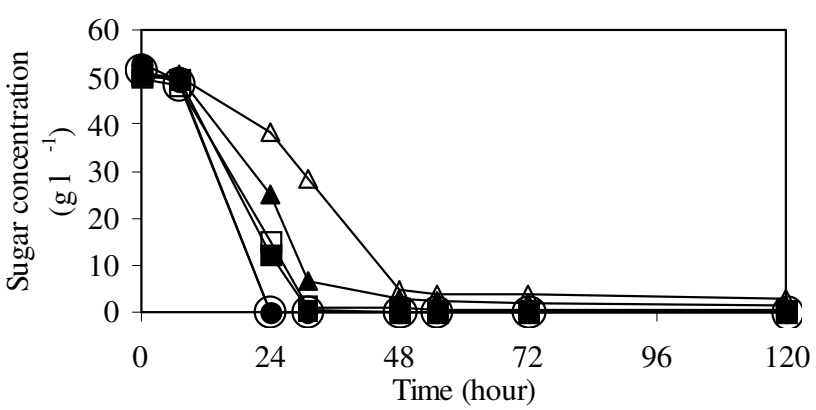


Figure 5.18 **a.** Specific rate of sugar utilization with CWP concentration **3b.** Specific rate of ethanol formation with CWP concentration



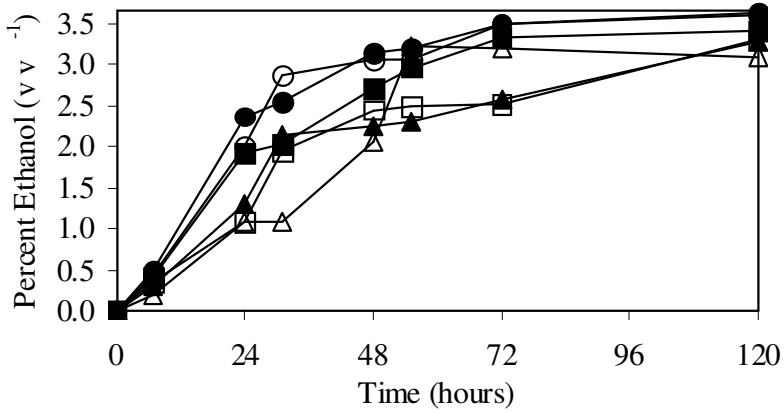


Figure 5.19 **a.** Variation of sugar concentration with time, **4b.** Variation of percent ethanol concentration with time.Biomass concentration (mg l^{-1}):

Δ 170,▲ 340, □ 510, ■ 680, ○ 850, ● 1020

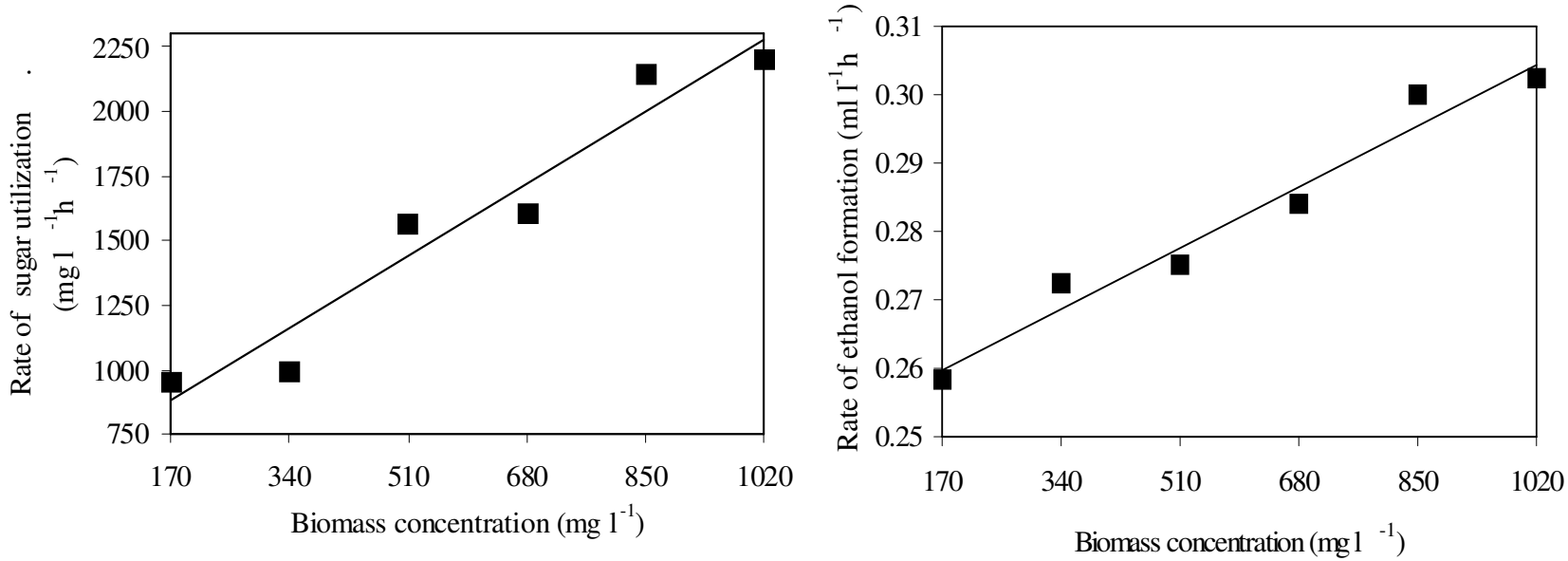


Figure 5.20 **a.** Variation of sugar utilization rate with initial biomass concentration, **b.** Variation of ethanol formation rate with initial biomass concentration

5.1.6 Kinetic Modelling and Estimation of the Kinetic Constants

The following kinetic model results were used to describe the initial rate of sugar (substrate) utilization for batch fermentation of CWP to ethanol using *K. marxianus* DSMZ-7239.

Theoretical background on ethanol fermentation by batch operation was presented in section 4.1. The equations derived in that section were used for determination of the kinetic constants. When the experimental data (Figure 5.18 a) for sugar concentrations below 78 gl^{-1} was plotted in form of $1/R_{so}$ versus $1/S_o$ the following constants were found for R_m and K_s.

R_m = 10.25 gS l^{-1} h^{-1}, K_s = 738 g l^{-1} and k = 20.5 g S gX^{-1} h^{-1} since X_o was 0.5 g l^{-1}.

Therefore, eqn 2 takes the following form for S_o< 78 g l^{-1}.

$$R_{SO} = \frac{k X_o S_o}{K_S + S_o} = \frac{20.5 Xo S_o}{738 + S_o} \qquad \text{(Eqn 2 b)}$$

Extremely high value of K_s indicated that the kinetics can be approximated to the first order. Since S_o is much lower than K_s (i.e, $S_o/K_s < 0.1$) for S_o < 78 g l^{-1}, then S_o in the denominator may be neglected to yield

$$R_{so} = (k/ K_s) X_o S_o = 0.0278 X_o S_o \qquad \text{(Eqn 2 c)}$$

For sugar concentrations above 78 g l^{-1}, substrate inhibition was observed as presented in Figure 5.18 a. Therefore at high substrate concntrations (S_o> 78 g l^{-1}) only the inhibition term was considered and the eqn 1 was approximated to the following expression.

$$R_{so} = R_{sm} \frac{K_{SI}}{K_{SI} + S_o} = k' Xo \frac{K_{SI}}{K_{SI} + S_o} \qquad \text{(Eqn 15)}$$

In double reciprocal form, Eqn 15 takes the following form,

$$\frac{1}{R_{SO}} = \frac{1}{R_{sm}} + \frac{S_o}{R_{sm} K_{SI}} \qquad \text{(Eqn 15 a)}$$

when the experimental data (Figure 5.18 a) for $S_o > 78$ g l^{-1} (Eqn 15 a) was plotted in form of $1/R_{so}$ versus S_o, the following constants were obtained from the slope and intercept of the line.

$R_{sm} = 1.425$ g S l^{-1} h^{-1}, $K_{SI} = 125$ g l^{-1} , k' = 2.85 gS gX^{-1} h^{-1} since X_o was 0.5 g l^{-1}.

Then, Eqn 15 takes the following form,

$$R_{so} = k' X_o \frac{K_{SI}}{K_{SI} + S_o} = 2.85 X_o \frac{125}{125 + S_o} \qquad \text{(Eqn 15 b)}$$

R_{so} values for $S_o < 78$ g l^{-1} and $S_o > 78$ g l^{-1} were estimated using Eqn's 2 b and 15 b, respectively.

Table 5.1 summarizes the experimental and the predicted values of R_{so} for all sugar concentrations tested. Good agreement between the predicted and the experimental values of R_{so} values indicated accuracy of the kinetic constants and the validity of the rate expressions for the experimental conditions used.

Table 5.1 Experimental and the predicted rate data used for kinetic modelling. $X_o = 0.5$ g l^{-1}

S_o (g l^{-1})	$1/S_o$	$R_{so, exp}$ (g $l^{-1}h^{-1}$)	$1/R_{so}$	$R_{so,pred}$ (gS $l^{-1}h^{-1}$)
26	0.0385	0.350	2.86	0.353 (eqn.2b)
52	0.0192	0.675	1.48	0.674 (eqn 2b)
78	0.0128	1.00	1.00	0.98 (eqn 2b)
104	0.0096	0.79	1.25	0.78 (eqn 3b)
130	0.0077	0.70	1.43	0.70 (eqn 3b)
156	0.0064	0.64	1.54	0.633 (eqn 3b)

5.2 Fed-Batch Experiments

Effects of feed CWP content or sugar loading rate on sugar conversion and ethanol formation was investigated in fed-batch experiments.Volume of the fermentation media increased linearly with time (Vo = 1 l) since the flow rate of the CWP solution was kept constant at 0.084 l h^{-1} throughout the experiments. Sugar concentrations in the fermenter were always below those of the control experiments because of the sugar utilization by the yeast cells. Figure 5.21 depicts variations of total soluble sugar, ethanol, biomass concentrations and also pH and ORP with time in control and experimental fermenter for the feed sugar concentration of 58 ± 2 g l^{-1} during the five-cycle fed-batch experiments. As shown in Figure 5.21 a, soluble sugar concentrations during the first two fed-batch experiments were close to the control experiments indicating insignificant sugar utilization. However, sugar utilization improved for the last three cycles yielding considerably lower sugar concentrations in the experimental fermenter as compared to the control fermenter. The effluent sugar concentration at the end of the fifth-cycle was nearly 1.93 g l^{-1} when the feed sugar was 56.15 g l^{-1} yielding nearly 97% sugar utilization. Variations of percent ethanol concentrations (%, v v^{-1}) with time during the five-cycle repeated fed-batch experiments are depicted in Figure 5.21 b. Not much ethanol was formed during the first two runs, since not much sugar was fermented. Ethanol formation increased with the third run in parallel to the sugar consumption and the final ethanol of nearly 3.72% (v v^{-1}) was obtained at the end of the fifth-run. The ethanol yield at the end of the fifth-run was calculated as approximately $Y_P/_S$ = 0.61 g EtOH g^{-1} sugar which is very close to the theoretical yield of 0.54 g E g $lactose^{-1}$. Variations of biomass concentrations with time during the five-cycle fed-batch experiments are depicted in Figure 5.21 c where the biomass concentrations represent the difference between the total solids contents of the feed and the fermenter media. Biomass concentrations increased with time for the first three cycles and then remained constant indicating quasi-steady state conditions. The growth yield coefficient at the end of the operation was found to be Yx/s = 0.16 gX gS^{-1} which is close to the theoretical predictions of 0.12 gX g $lactose^{-1}$. The difference may be because of approximate determinations of biomass concentrations in the CWP solution because of the presence of solid substrates (CWP particles) in the medium. Figure 5.21 d and

Figure 5.21 e depict variations of pH and ORP with time during the course of repeated fed-batch experiments. pH increased from 4.5 to 4.7 during the first two cycles which then decreased gradually and reached a steady level of 4.2 at the end of the last two cycles indicating quasi steady-state conditions. Similar to pH variations, ORP of the fermentation medium increased from -200 mV to nearly -150 mV during the first cycle which then decreased gradually and reached a steady level of -300mV at the end of the last two cycles indicating quasi steady-state conditions.

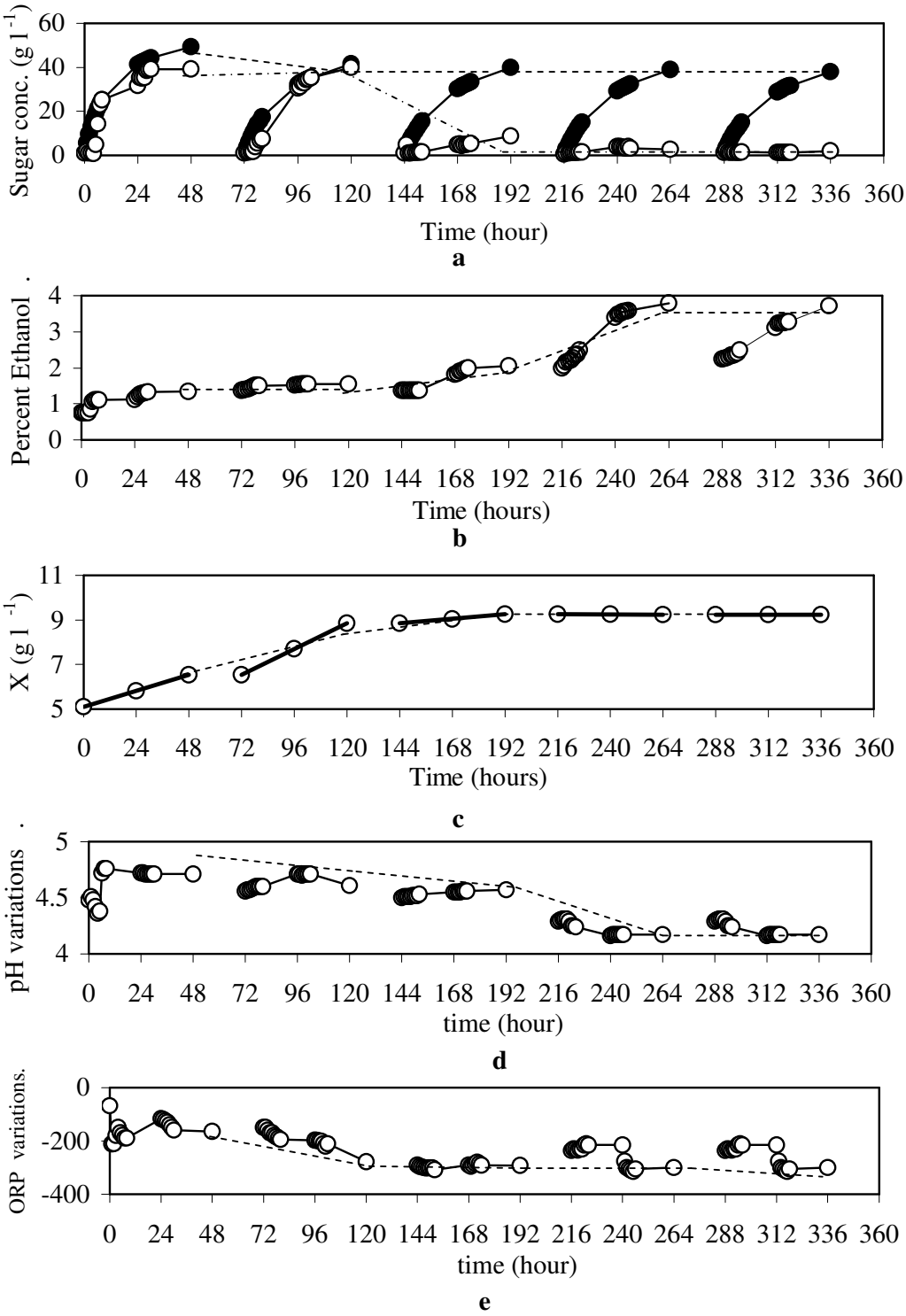


Figure 5.21 Fed-batch experiments with CWP containing 50 g l^{-1} total sugar. Variations of (a) sugar concentration with time, ●Control, o Experimental, (b) ethanol concentration with time (c) biomass concentration with time, (d) pH with time, (e) ORP with time; Q=0.084 l h^{-1}, 28°C, pH=5

Similar graphs were established for different feed sugar concentrations. Figure 5.22 depicts variations of total soluble sugar, ethanol, biomass concentrations and also pH and ORP variations with time in control and experimental fermenter for feed sugar concentration of 110 ± 5 g l^{-1} during the five-cycle fed-batch experiments. As shown in Figure 5.22 a, soluble sugar concentrations in the experimental fermenter were always lower than those of the control due to effective sugar utilization by the yeast cells. Soluble sugar concentrations at the end of each cycle decreased steadily and reached 12 ± 2 g l^{-1} for the last three cycles. The effluent sugar concentration at the end of the fifth-cycle was nearly 12.7 g l^{-1} when the feed sugar was 115.2 g l^{-1} yielding nearly 89% sugar utilization. Figure 5.22 b depicts variations of percent ethanol concentrations (%, v v^{-1}) with time during the five-cycle repeated fed-batch operation. Ethanol concentration increased from 0.9% to 3.24% at the end of the first-cycle which further increased with continuing operation and reached 6.8% at the end of the fifth-cycle. The ethanol yield coefficient at the end of the fifth-run was approximately $Y_{p/s}$ = 0.57 g EtOH g^{-1} sugar which is very close to the theoretical yield of 0.54 g E g $lactose^{-1}$. Variations of biomass concentrations with time during the five-cycle fed-batch experiments are depicted in Figure 5.22 c where the biomass concentrations represent the difference between the total solids contents of the feed and the fermenter media. Biomass concentrations increased gradually with time and reached 8.26 gX l^{-1} at the end of the fifth-cycle. The growth yield coefficient at the end of the operation was found to be $Y_{x/s}$ = 0.085 gX gS^{-1} which is lower than the theoretical prediction of 0.12 gX gS^{-1}. Low experimental growth yield coefficient may be because of reduced growth due to high osmotic pressure at high sugar concentrations. Figure 5.22 d and Figure 5.22 e depict variations of pH and ORP with time during the course of repeated fed-batch experiments. pH increased from 4.1 to 4.65 at the end of each cycle and was almost constant for the last three cycles indicating quasi steady-state. Similarly, ORP of the fermentation medium decreased from -150 mV to nearly -200 mV at the end of the first-cycle which further decreased and reached a steady level of -240mV at the end of the fifth-cycle indicating sustained anaerobic conditions throughout the operation.

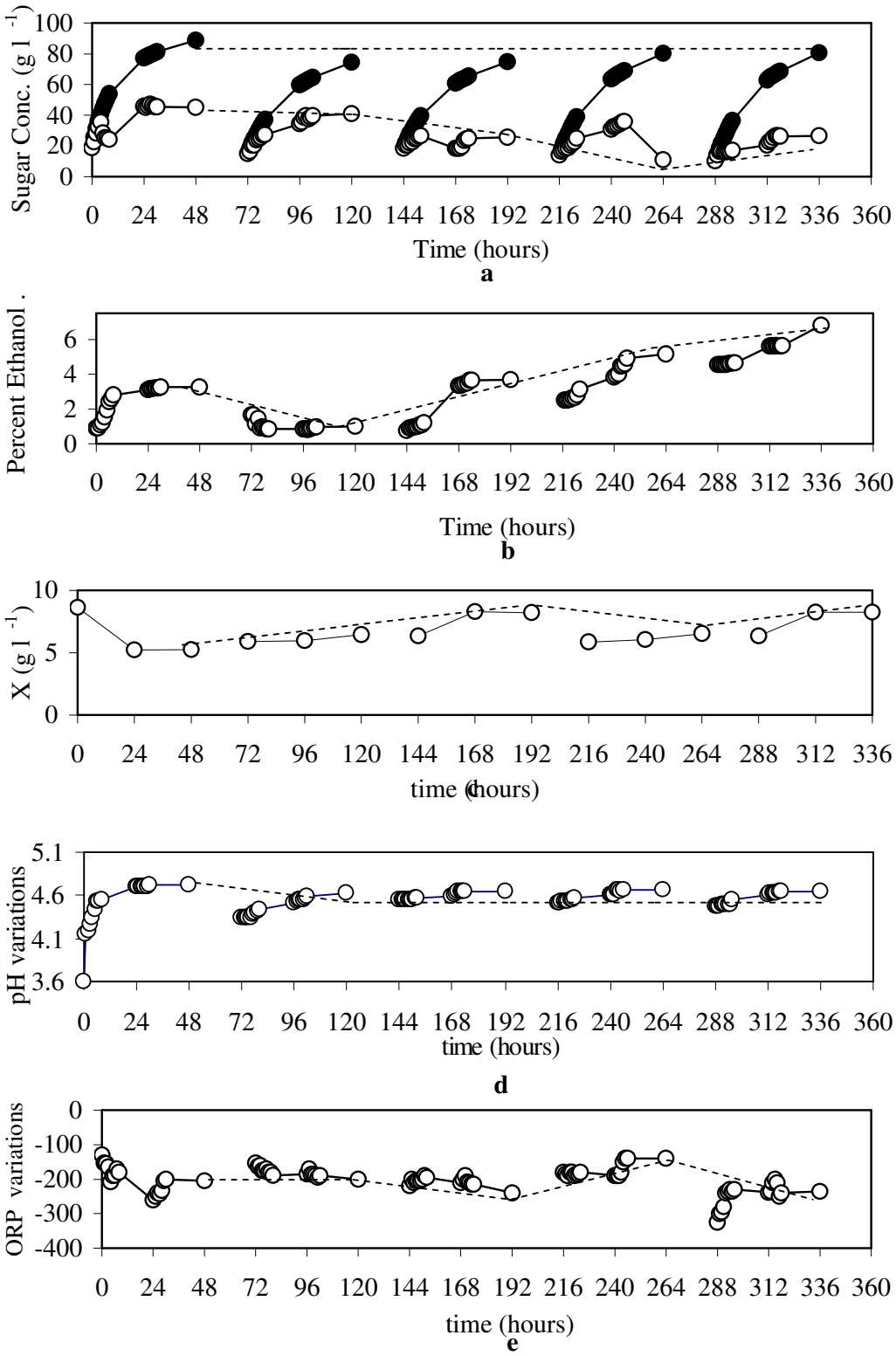


Figure 5.22 Fed-batch experiments with CWP containing 100 g l^{-1} total sugar. Variations of(a) sugar concentration with time, ●Control, ○ Experimental, (b) ethanol concentration with time (c) biomass concentration with time, (d) pH with time, (e) ORP with time; Q=0.084 l h^{-1}, 28^{o}C, pH=5

Figure 5.23 shows variations of total soluble sugar, ethanol, biomass concentrations and also pH and ORP variations with time in control and experimental fermenters for the feed sugar concentration of 155± 5 g l^{-1} during the five-cycle fed-batch experiments. Soluble sugar concentrations in the experimental fermenters were always lower than those of the control fermenter. As depicted in Figure 5.23 a, difference in sugar concentrations of the experimental and the control fermenters or sugar utilization increased with the increasing number of cycles due to increased cell concentrations. The effluent sugar concentration at the end of the fifth-cycle was nearly 65.9 g l^{-1} when the feed sugar was 152.7 g l^{-1} yielding nearly 57% sugar utilization. Figure 5.23 b depicts variations of percent ethanol concentrations (%, v/v) with time during the five-cycles. Ethanol formation increased in parallel to the sugar utilization from 4.2% at the beginning of the first cycle to nearly 6.8% (v/v) at the end of the fifth-cycle. The ethanol yield at the end of the fifth-run was calculated as approximately Yp/s = 0.62 g EtOH g^{-1} sugar which is a little above the theoretical yield of 0.54 g E g^{-1} lactose. Variations of biomass concentrations with time during the five-cycle fed-batch experiments are depicted in Figure 5.23 c where the biomass concentrations represent the difference between the total solids contents of the fermenter and the feed media. Biomass concentrations decreased from 9.4 g l^{-1} at the beginning of the first-cycle to 8.6 g l^{-1} at the end of the fifth-cycle due to adverse effects of osmotic pressures of high sugar concentrations. Biomass concentrations at the end of the last two cycles were almost the same indicating the quasi steady-state conditions. The growth yield coefficient at the end of the fifth-cycle was found to be approximately $Y_{x/s}$ = 0.1gX gS^{-1} which is close to the theoretical predictions of 0.12 gX g lactose^{-1}. Figure 5.23 d and Figure 5.23 e depict variations of pH and ORP with time during the course of repeated fed-batch experiments. pH increased slightly from 4.55 to 4.65 at the end of the third-cycle and remained constant for the last two cycles indicating the quasi steady-state conditions. Unlike pH variations, ORP of the fermentation medium decreased from -300 mV to nearly -340 mV for the last two cycles. ORP values also reached a steady level for the last two cycles. When compared with the results obtained with a feed sugar content of 50 g l^{-1}, the biomass yield coefficient ($Y_{x/s}$) decreased, but the ethanol yield coefficient increased ($Y_{p/s}$) when the feed sugar concentration was increased to

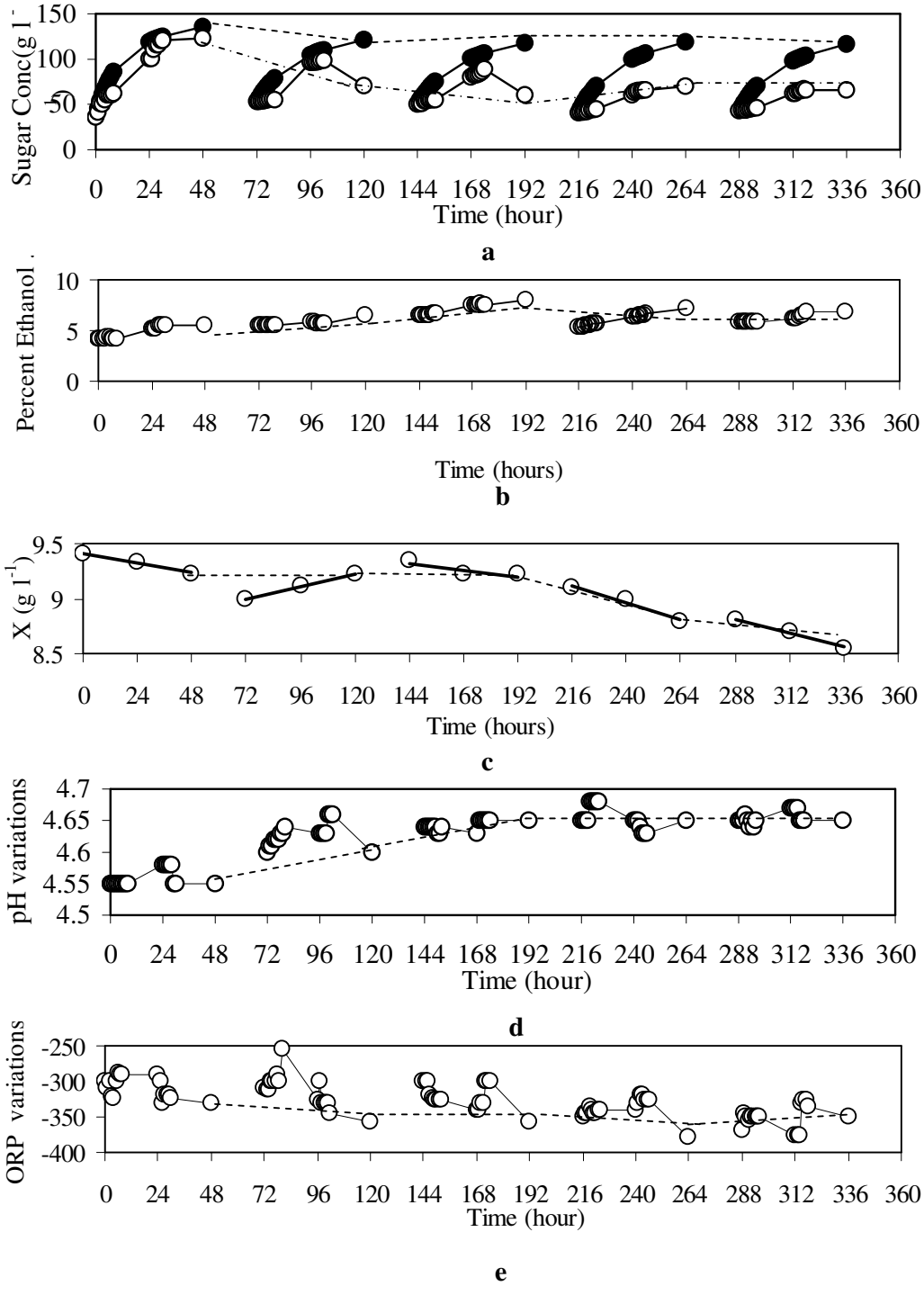


Figure 5.23 Fed-batch experiments with CWP containing 150 g l^{-1} total sugar. Variations of (a) sugar concentration with time, ●Control, o Experimental, (b) ethanol concentration with time (c) biomass concentration with time, (d) pH with time, (e) ORP with time; Q=0.084 l h^{-1}, 28^{o}C, pH=5

150 g l^{-1}. Apparently, at high sugar concentrations biomass concentrations decreased due to high osmotic pressure, but the energy produced from sugar metabolism was channeled to ethanol formation rather than biomass.

When the feed sugar concentration was further increased to 200 g l^{-1}, sugar utilization decreased considerably due to high osmotic pressure caused by high sugar concentrations. Soluble sugar concentrations in the experimental fermenter were slightly lower than those of the control fermenter indicating ineffective utilization of sugar by the yeast cells at high feed sugar concentration of 200 g l^{-1}. Difference in sugar concentrations of the experimental and the control fermenters were in the order of 10-15 g l^{-1}. The effluent sugar concentration at the end of the fifth-cycle was nearly 155.7 g l^{-1} when the feed sugar was 200 g l^{-1} yielding nearly 22.5% sugar utilization. Ethanol formation increased in parallel to the sugar utilization from 3.45% at the beginning of the first-cycle to nearly 6.5% (v v^{-1}) at the end of the fourth and further to 5.1% at the end of the fifth-cycle. The ethanol yield at the end of the fifth-run was approximately $Y_{p/s}$ = 0.89 g EtOH g^{-1} sugar which is considerably above the theoretical yield of 0.54 g E gS^{-1}. The reason for this may be release of intracellular ethanol to the medium upon cell disintegration due to high osmotic pressure at high sugar concentrations above 150 g l^{-1}. In fact, sugar concentrations in the fermenter were well above 120 g l^{-1} during the operation when the fed sugar was 200 g l^{-1}. Biomass concentrations decreased from 8.5 g l^{-1} at the beginning of the first-cycle to 2.7 g l^{-1} at the end of the fifth-cycle due to adverse effects of high sugar concentrations causing high osmotic pressure. Biomass concentrations at the end of the last two cycles were almost the same indicating the quasi steady-state conditions. The growth yield coefficient at the end of the fifth-cycle was found to be approximately Yx/s = 0.05 gX gS^{-1} which is considerably lower than that of the theoretical predictions of 0.12 gX g^{-1} lactose again probably due to cell disruption by high osmotic pressure at high sugar concentrations. pH increased slightly from 4.55 to 4.65 at the end of the third-cycle and remained constant for the last two cycles indicating the quasi steady-state conditions. Unlike pH variations, ORP of the fermentation medium decreased from -300 mV to nearly -350 mV for the last three cycles indicating steady-state conditions When compared with the results obtained with a feed sugar content of 50 and 150 g l^{-1}, the biomass

yield coefficient ($Y_{x/s}$) decreased, but the ethanol yield coefficient increased ($Y_{p/s}$) when the feed sugar concentration was increased to 200 g l^{-1}. Apparently, at high sugar concentrations biomass concentrations decreased but the ethanol concentration increased. The reason of ethanol yield increases lies on the ethanol which was adsorbed by the settled organisms at the end of each cycle. The procedure of every fed batch cycle finished with settling the organisms and harvesting the supernatant to prepare the system for the next cycle. When the system was operated for the next cycle the adsorbed ethanol concentration disorbed, and increased the overall ethanol concentration of the system.

Variations of percent sugar utilization and ethanol formation at the end of the fifth-cycle with the feed sugar concentrations are depicted in Figure 5.24. Percent sugar utilizations decreased from 95% to 22% when the feed sugar concentration increased 50 to 200 g l^{-1} due to high sugar loading rates. Percent ethanol concentrations increased from 3.33%(v v^{-1}) to 7.97% when the feed sugar was increased from 50 to 125 g l^{-1}. Further increases in the feed sugar to 200 g l^{-1} resulted in 5.1 % ethanol formation due to lower percent sugar utilizations at high feed sugar concentrations. The optimal feed sugar concentration was 125 g l^{-1} yielding the highest percent ethanol formation (7.97%, v v^{-1}).

Variations of growth yield ($Y_{x/s}$) and product yield coefficient ($Y_{P/S}$) with the feed sugar concentration are depicted in Figure 5.25. The growth yield coefficient decreased from 0.16 gX gS^{-1} to 0.05 gX gS^{-1} when the feed sugar concentration was increased from 50 g l^{-1} to 200g l^{-1} due to inhibited growth at high feed sugar concentrations. The product yield coefficients were around 0.6-0.65 g P g S^{-1} for the feed sugar contents below 150 g l^{-1} which increased to 0.89 gP gS^{-1} for the feed sugar of 200 g l^{-1}. The reason for high product yield coefficients at high sugar concentrations is probably due to intracellular ethanol release because of cell disruption at by high osmotic pressures at high sugar concentrations.

Figure 5.26 depicts variation of ethanol productivity (Q P_f, gE h^{-1}) at the end of the fifth-cycle with sugar loading rate (Q S_i, gS h^{-1}). Ethanol productivity increased with the sugar loading rate up to feed sugar concentration of 125 g l^{-1} (or loading rate of 10.5 g sugar h^{-1}), due to effective sugar utilization with simultaneous ethanol

formation. Productivity of ethanol decreased at sugar loading rates above 1.8 g S l^{-1} h^{-1} to 0.77 and 0.75 g E l^{-1} h^{-1} for the feed sugar concentrations of 150-200 g l^{-1}, respectively. Further increases in sugar loading rates caused decreases in ethanol productivity due to adverse effects of high osmotic pressure caused by high sugar

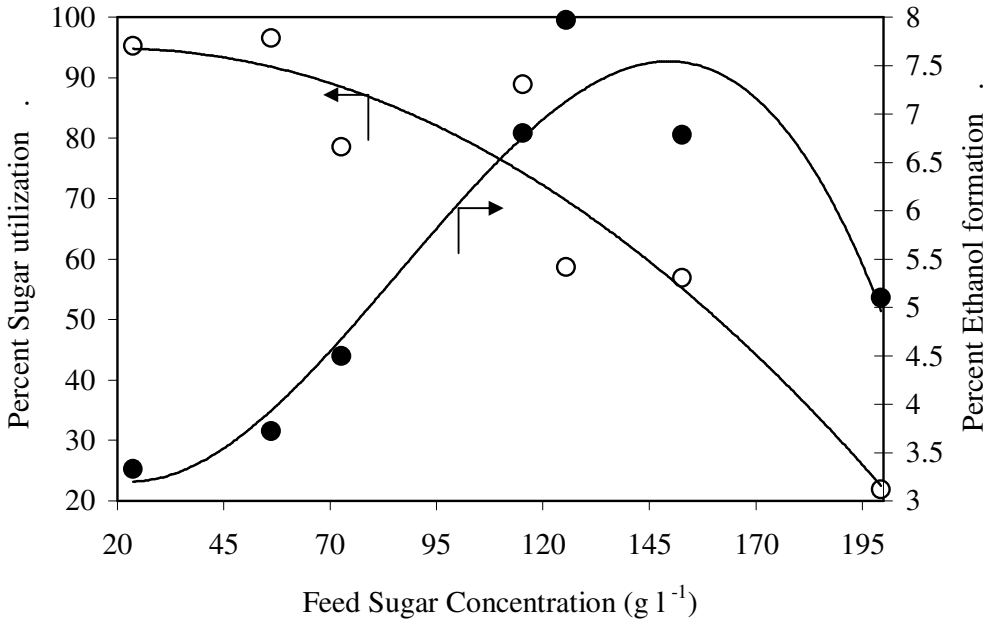


Figure 5.24. Variations of percent sugar utilization and percent ethanol formation with the feed sugar concentration.; Q=0.084 l h^{-1}, 28°C, pH=5

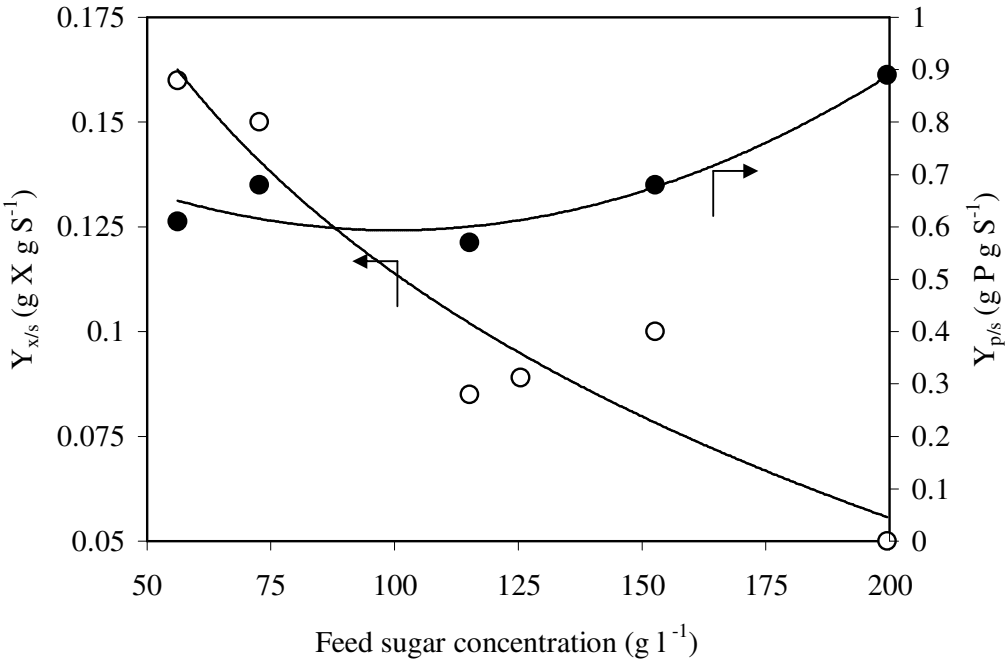


Figure 5.25 Variations of the growth ($Y_{x/s}$, gX/gS) and the product (ethanol, $Y_{p/s}$, gE/gS) yield coefficients with the feed sugar concentration; Q=0.084 l h^{-1}, 28°C, pH=5

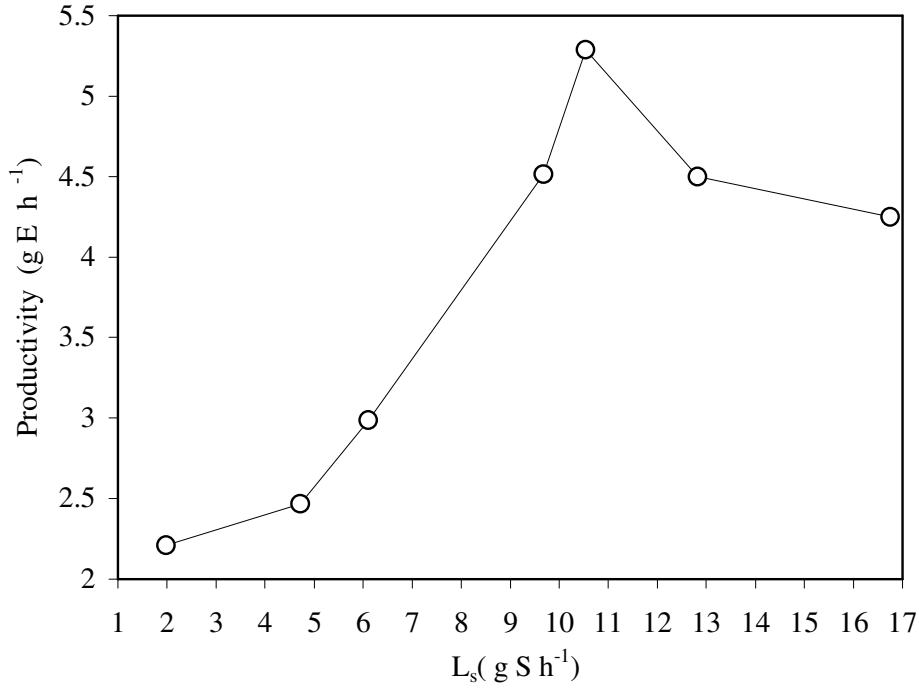


Figure 5.26 Variation of ethanol productivity ($Q.E_f$) at the end of the fifth-cycle with the sugar loading rate ($Q\ S_i$); Q=0.084 l h^{-1}, 28°C, pH=5

loadings. Optimal sugar loading rate yielding the highest ethanol productivity was10.5 g S h^{-1} yielding ethanol productivity of 5.3 g EtOH h^{-1}.

Effects of feed CWP content or sugar loading rate on sugar conversion and ethanol formation have been investigated in repeated fed-batch experiments. Figure 5.27 depicts an example of typical variations of important process variables with time for the fed-batch experiment with the feed sugar of 125 g l^{-1} and the feed flow rate of 0.084 l h^{-1}. Media volume and total amount of biomass in the fermentor increased with time linearly as expected theoretically. Sugar concentration in the control fermentor increased with time due to accumulation of sugar in the absence of organisms. However, in the experimental fermentor sugar content increased slightly. Percent sugar conversion based on the difference in sugar concentrations in the control and the experimental fermentor increased with time as a result of increases in total biomass in the fermentor. Ethanol concentration also increased with time in the fermentor.

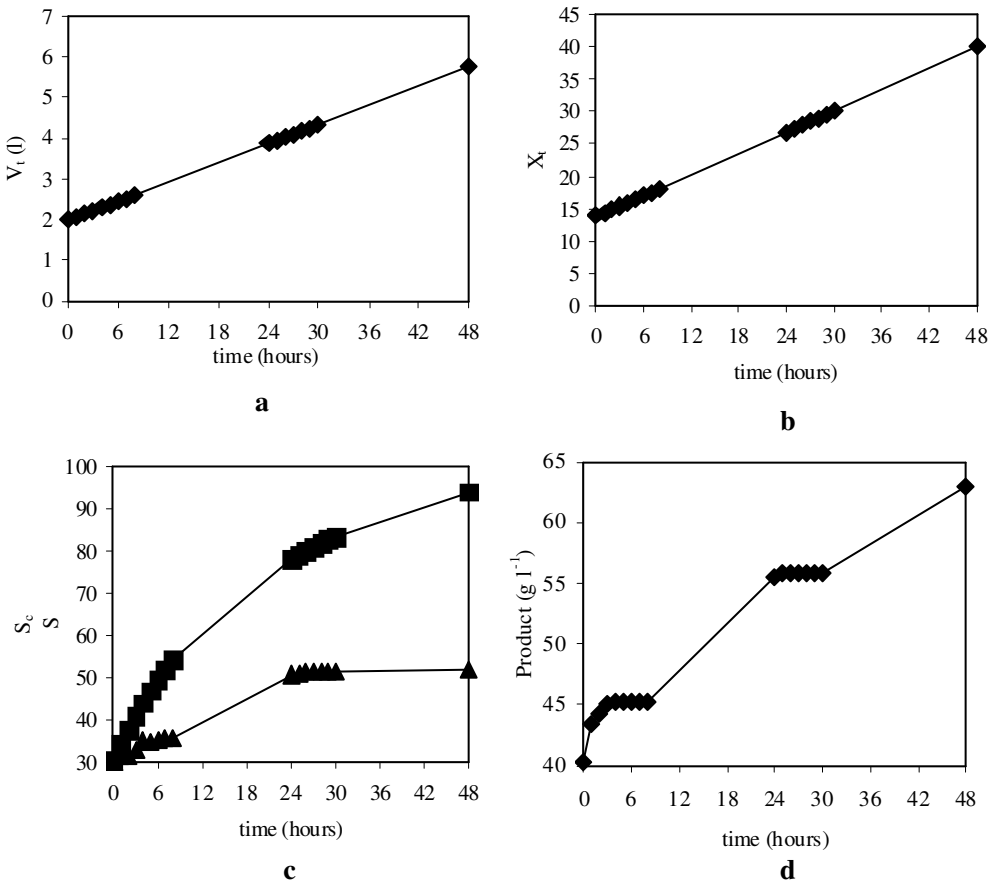


Figure 5.27 Variation of process variables with time in fed-batch operation for feed sugar concentration of 125 g l^{-1} and feed flow rate of 0,084 l h^{-1} (a) Media volume in the fermentor; (b) total biomass in fermentor; (c) Sugar concentration: control (■), experimental (▲) (d) Product formation; Q=0.084 l h^{-1}, 28°C, pH=5

5.3 Continuous Fermentation Experiments

5.3.1 Effects of Hydraulic Residence Time

5.3.1.1 Experimental Results

Continuous experiments were performed at seven (7) different HRT levels between 12.5 and 60 hours which were established by changing the feed flow rate while keeping the fermentation volume at 3 litre constant level. Figure 5.28 depicts variation of the effluent total sugar concentration and percent sugar utilization with the HRT for a constant feed sugar content of S_o = 100 ± 5 g l^{-1}. The effluent sugar

contents decreased and percent sugar utilization increased with increasing HRT. The effluent sugar decreased from 95 g l^{-1} (S_o = 110 g l^{-1}) to 15 (S_o= 99.6 g l^{-1}) and percent sugar utilization increased from 15 to 86% when the HRT increased from 12.5 to 60 hours. Variations of ethanol concentrations in the fermenter and the ethanol productivity (DP) with the HRT are shown in

Figure 5.29. Ethanol concentration increased with HRT due to higher percent sugar utilizations at high HRT levels. Ethanol productivity increased with HRT and reached to the highest level of 0.745 g E l^{-1} h^{-1} at an HRT of 43.2 h and decreased with further increases in HRT. The optimum HRT maximizing the ethanol productivity was found to be 43.2 h (D = 0.023 h^{-1}) where the specific growth rate was minimum.

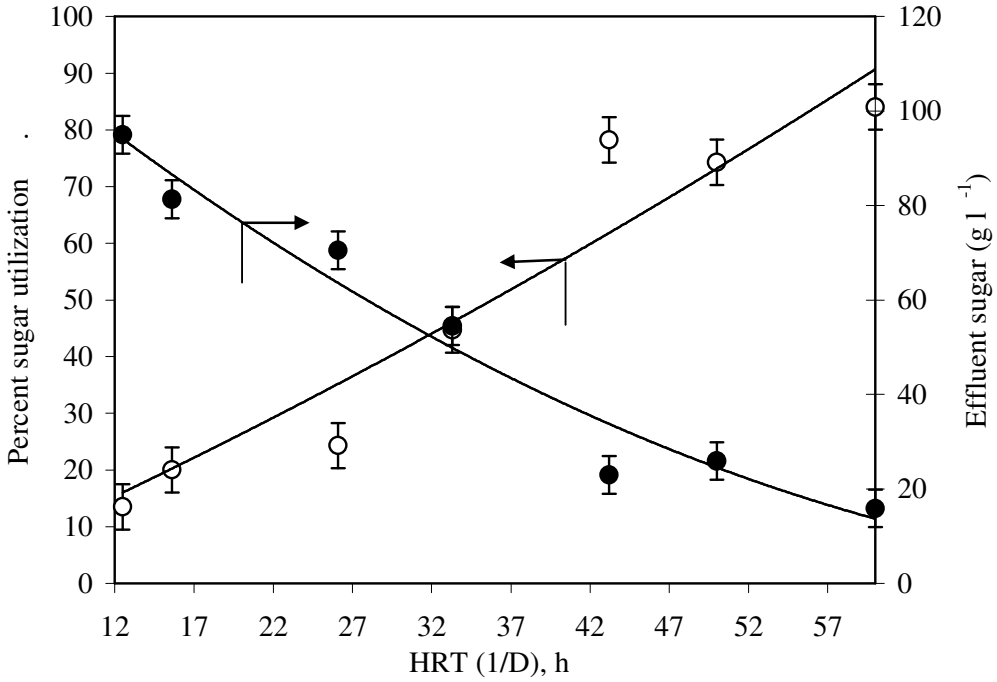


Figure 5.28 Variation of effluent sugar and percent sugar utilization with HRT (1/D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

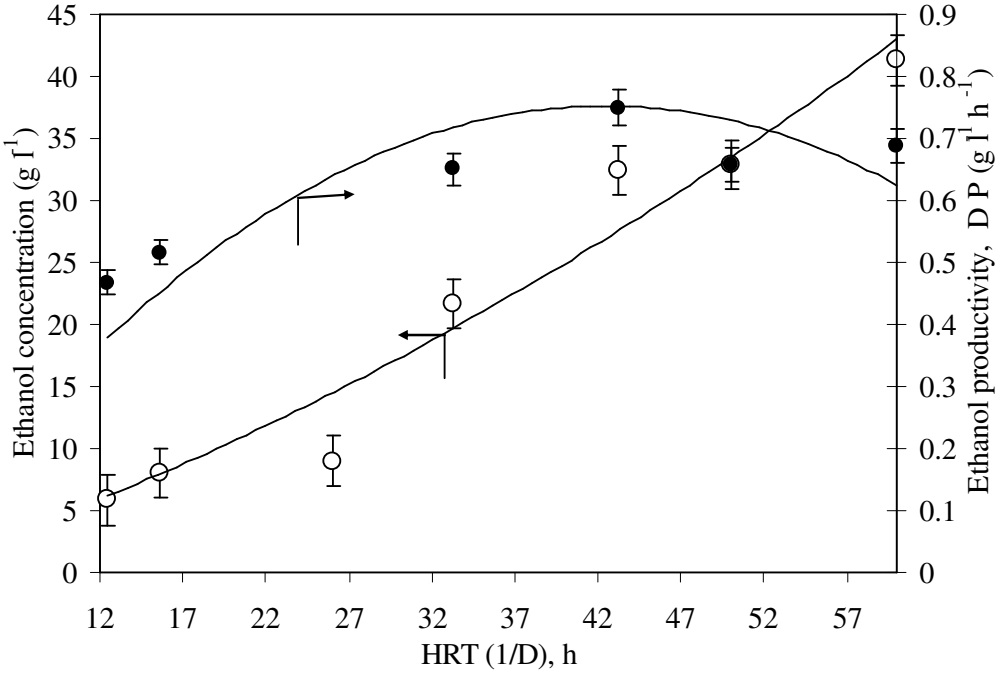


Figure 5.29 Variation of ethanol concentration and ethanol productivity (DP) with HRT (1/D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

Figure 5.30 depicts variation of biomass (yeast) concentration and the biomass productivity with HRT at the steady-state. Biomass concentration increased with increasing HRT because of larger percent utilization of sugar at high HRT levels. Biomass productivity was maximum at an HRT of 15.6 hours which decreased further and became minimum at a HRT of 43.2 hours where the ethanol productivity was maximum. Since the objective was to maximize the ethanol productivity and minimize the biomass productivity, operation at an HRT of 43.2 hours is recommended.

Variations of the ethanol ($Y_{P/S}$) and the growth ($Y_{X/S}$) yield coefficients with the HRT are depicted in Figure 5.31. The ethanol yield coefficient was almost constant around 0.4 gE g^{-1}S up to HRT of 43.2 h which increased to 0.496 gE g^{-1}S with further increases in HRT to 60 h. The theoretical ethanol yield from lactose is 0.54 gE g^{-1}lactose. At low HRT or high dilution rates where the specific growth rates are high, most of the sugar was used for growth yielding low product yield coefficients. At high HRT or low dilution rates where the specific growth rates are low, most of the sugar was converted to ethanol rather than biomass resulting in high product yield coefficients. The growth yield coefficients ($Y_{X/S}$) decreased with increasing HRT (or decreasing dilution rate and specific growth rate) and reached the lowest value at HRT of 43.2 hours where the ethanol yield was maximum. Further increases in HRT resulted in increases in the growth yield coefficient due to lower ethanol productivities at HRT levels above 43.2 h.

Specific rate of sugar utilization (q_s) increased with dilution rate (D) as depicted in Figure 5.32. High growth rates at high dilution rates (or low HRT levels) yielded high sugar utilization rates since the growth rate is related with substrate utilization rate by the yield coefficient, $Y_{x/s}$. The highest q_s value 0.42 gS g^{-1}X h^{-1} was obtained at the lowest HRT of 12.5 h corresponding to the highest dilution rate. Similarly, variation of specific rate of ethanol formation (q_p) with dilution rate (D) is shown in Figure 5.33 where q_p increased with dilution rate almost linearly with a slope of approximately 1.75. Since ethanol formation is growth associated ($q_p = \alpha \mu$), high growth rates at high dilution rates resulted in high specific ethanol formation rates. The highest q_p value (0.165 gP g^{-1}X h^{-1}) was obtained at the lowest HRT of 12.5 h.

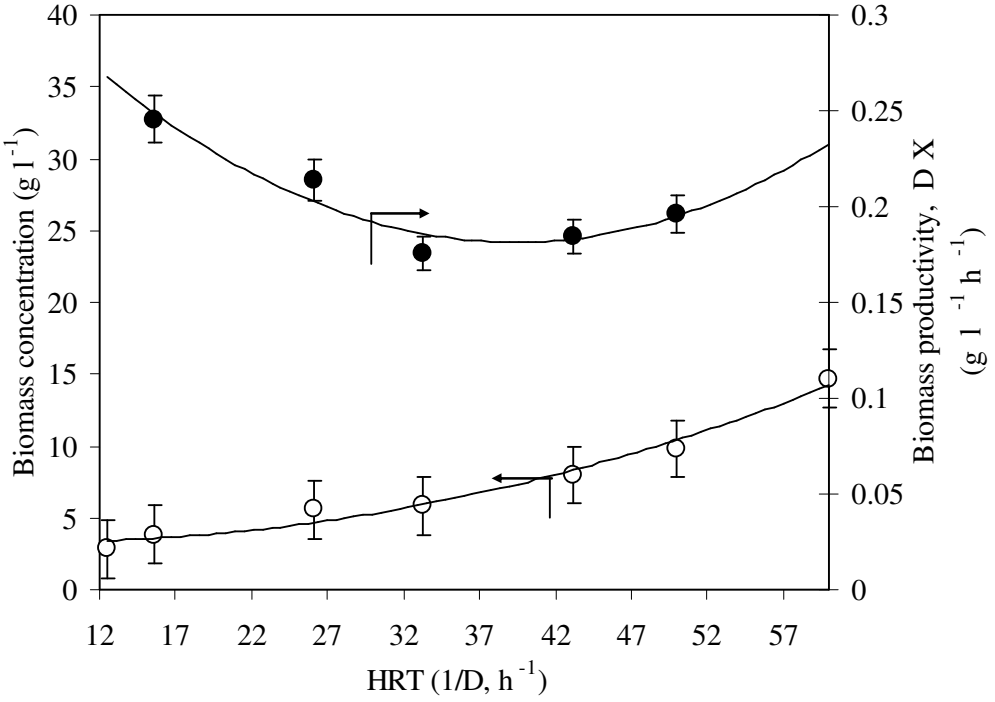


Figure 5.30. Variation of biomass (yeast) concentration and productivity (DX) with HRT (1/D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

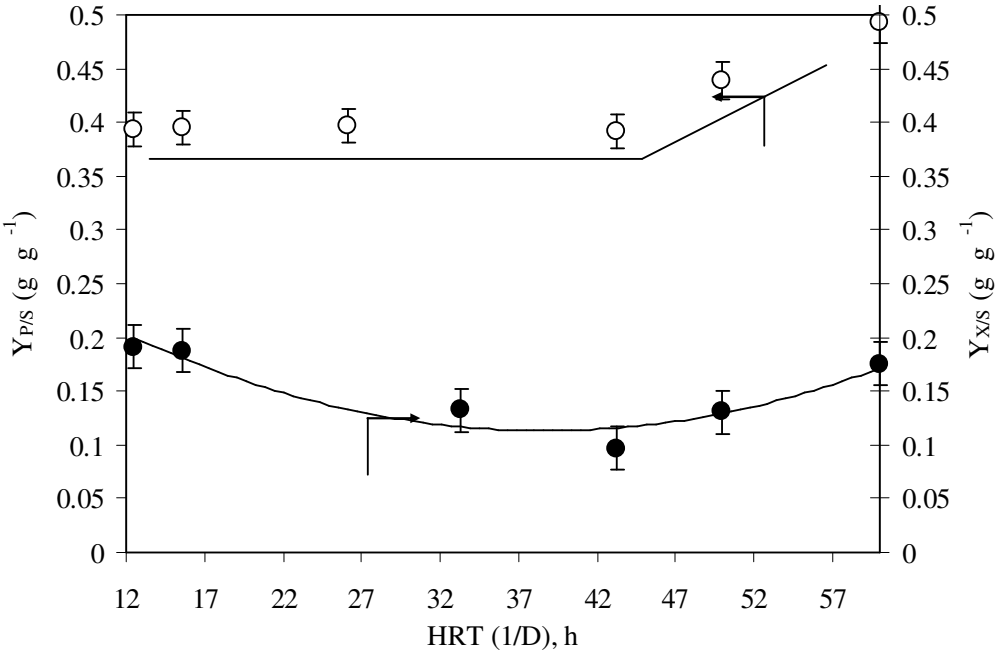


Figure 5.31 Variation of the apparent growth yield ($Y_{X/S}$) and product yield ($Y_{P/S}$) cooefficients with HRT (1/D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

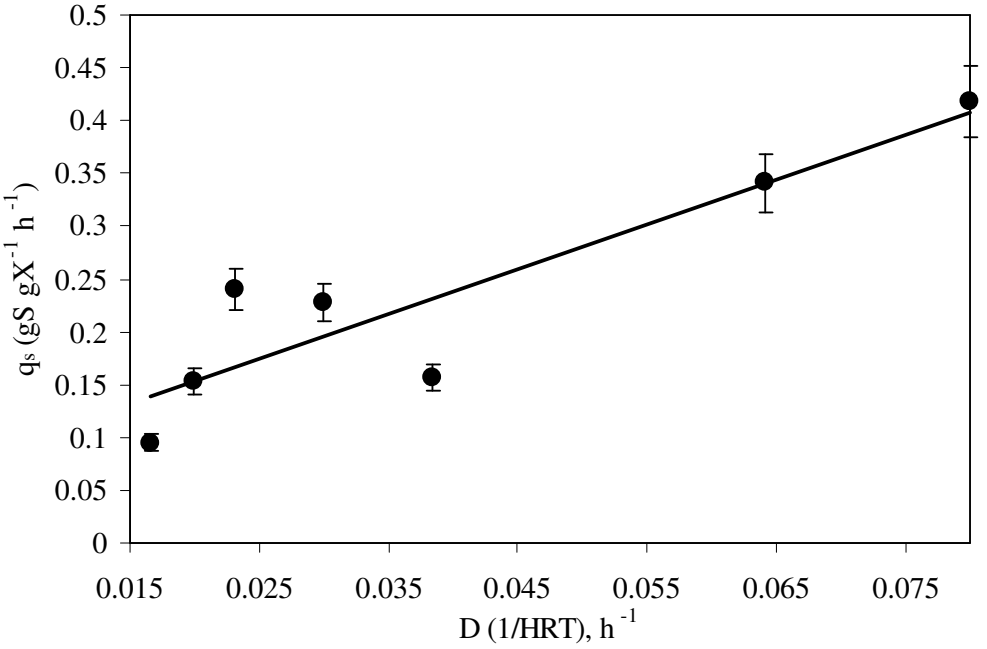


Figure 5.32 Variation of specific substrate utilization rate (q_s) with dilution rate (D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

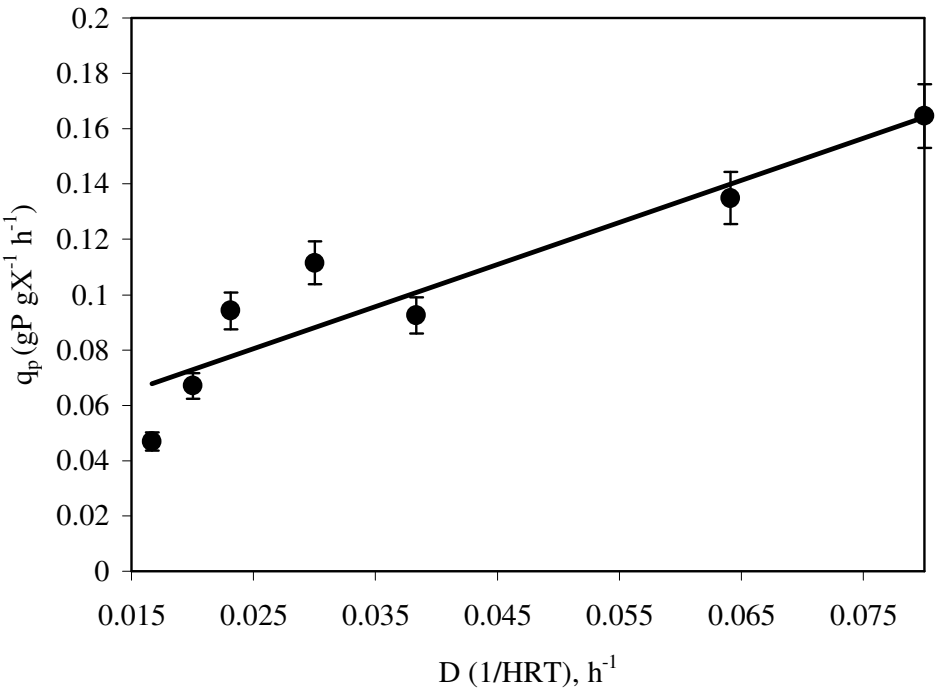


Figure 5.33 Variation of specific product (ethanol) formation rate (q_p) with dilution rate (D); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

5.3.1.2 Estimation of the Kinetic and Stoichiometric Coefficients

Theoretical background of continuous ethanol fermentation is presented in Section 4.3. The kinetic constants of the equations derived in that section were determined by using the experimental data. A plot of the experimental data in form of (P) versus X is depicted in Figure 5.34. From the slope of the best fit line the α value (or $Y_{P/X}$) was found to be 3.05 g P g^{-1}X (Eqn 11 b).

Eqn 10 d was used to estimate theY_M, b and α by using the experimental data obtained at different HRT's. The $Y_{P/S}$ value was taken as 0.42 gP g^{-1}S which was the average yield calculated from our experimental data. A STATISTICA 5.0 iteration program with Newton- Raphson approximation method was used for the estimation of the coefficients as follows,

$Y_M = 0.2$ gX g^{-1}S , $b = 0$, $\alpha = 3.16$ ($R^2 = 0.87$)

Since the maximum HRT was 60 h and the minimum sugar concentration at the steady-state was 15.25 g l^{-1}, the basal metabolism rate constant (b) was found to be negligible. Therefore the Eqn 9 c takes the following form with a negligible (b).

$1/D = 1/\mu_m + (K_s /\mu_m)\ (1/S)$ (Eqn 9 d)

A plot of 1/D versus 1/S yields a straight line with a slope of K_s /μ_m and y-axis intercept of $1/\mu_m$ (Figure 5.35). From the slope and intercept of the best fit line the following coefficients were obtained

$\mu_m = 0.094\ h^{-1}$, $K_s = 78.5$ g l^{-1} ($R^2 = 0.89$)

The Y_M value of 0.20 gX g^{-1}S was found to be the maximum growth yield coefficient in the absence of basal (endogenous) metabolism which is comparable with the literature values.

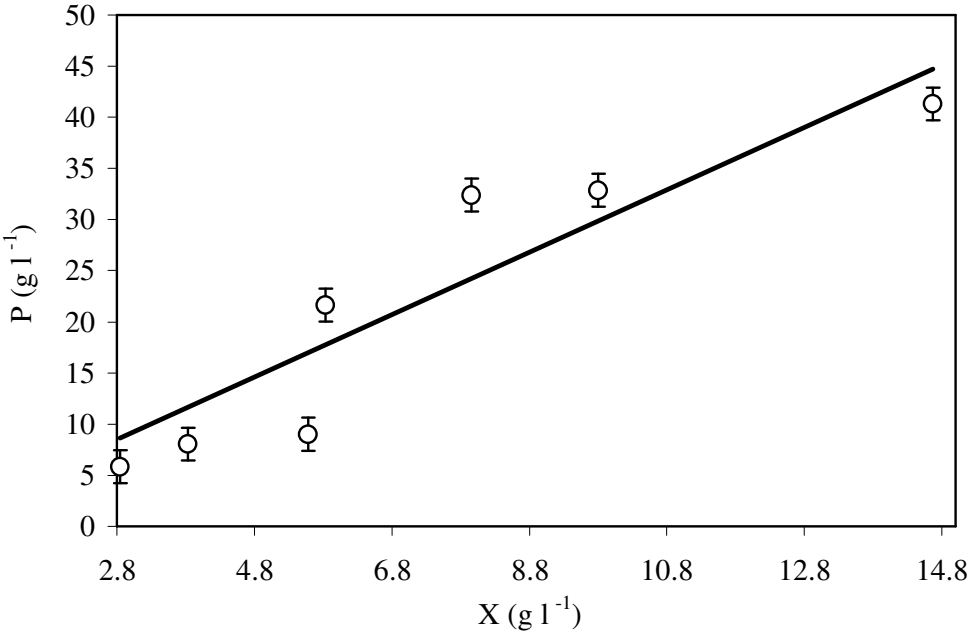


Figure 5.34 A plot of P (ethanol) versus X (yeast) concentrations to determine the coefficient α ($Y_{P/X}$); Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

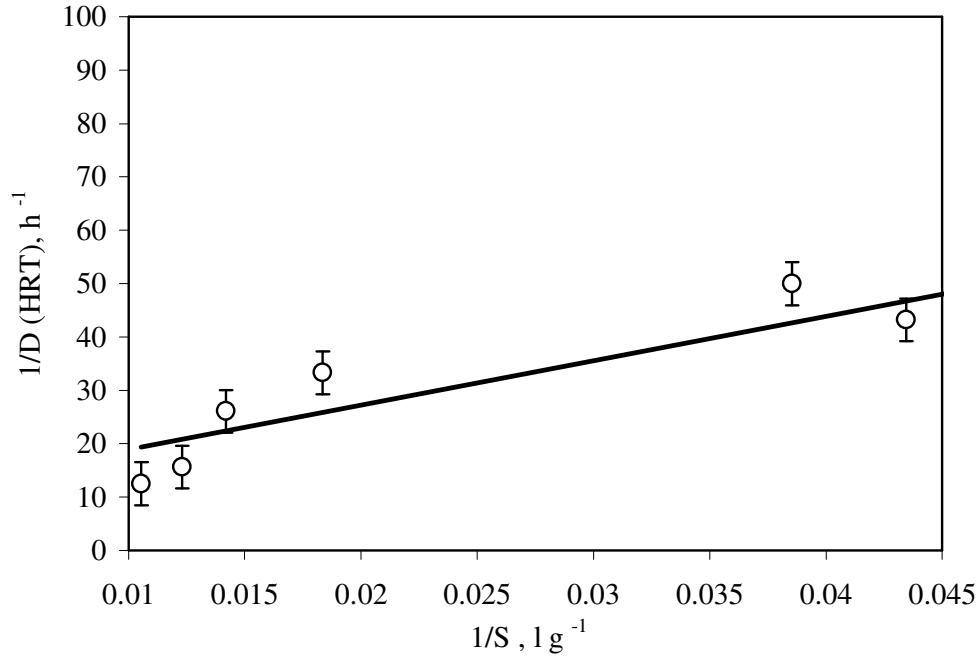


Figure 5.35 A plot of 1/D versus 1/S for determination of μ_m and K_s with negligible 'b'; Vt=3 l, So=100g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^{o}C

5.3.2 Effects of Feed Sugar Concentration

Continuous experiments were performed at six different feed sugar concentrations between 55 and 200 g l^{-1} at a constant HRT of 54 hours. Figure 5.36 depicts variation of the effluent total sugar concentration and percent sugar utilization with the feed sugar concentration. The effluent sugar increased and percent sugar utilization decreased with increasing feed sugar content due to adverse effects of high sugar concentrations on sugar utilization by the yeast cells. The effluent sugar increased from 15.6 g l^{-1}(S_o = 55 g l^{-1}) to 146.3 g l^{-1} (S_o= 200 g l^{-1}) and percent sugar utilization decreased from 71.6 to 26.6% when the feed sugar content increased from 55 to 200 g l^{-1}. Apparently high sugar concentrations and other dissolved solids increased the osmotic pressure of the fermentation broth which resulted in considerable activity loss in the yeast cells.

Variations of ethanol concentrations (P) and productivity (DP) with the feed sugar concentration are shown in Figure 5.37. Both final ethanol concentration (P) and productivity (DP) increased with the feed sugar content up to 100 g l^{-1} and reached maximum levels of 3.7% (v v^{-1}) and 0.54 gE l^{-1} h^{-1}, respectively. Further increases in the feed sugar content resulted in decreases in ethanol yield and productivity due to adverse effects of high osmotic pressure at high sugar concentrations. The optimal feed sugar content resulting in the highest ethanol yield and productivity was 100 g l^{-1} although the results obtained at 125 g l^{-1} feed sugar concentration were close to that obtained at 100 g l^{-1}. Ethanol concentration and the productivity decreased to 2% (v v^{-1}) and 0.29 gE l^{-1} h^{-1} when the feed sugar content was increased to 200 g l^{-1}.

Figure 5.38 depicts variation of biomass (yeast) concentration (X) and the biomass productivity (DX) with the feed sugar content at an HRT of 54 hour. Biomass concentration and productivity did not change significantly for the feed sugar concentrations between 55 and 125 g l^{-1}. However, further increases in the feed sugar content above 125 g l^{-1} resulted in considerable decreases in both biomass concentration and the productivity. Biomass concentration and the productivity decreased to 3.34 gX l^{-1} and 0.062 gX l^{-1} h^{-1} when the feed sugar content was increased 200 g l^{-1}.

Variations of the ethanol ($Y_{P/S}$) and the growth ($Y_{X/S}$) yield coefficients with the feed sugar content are depicted in Figure 5.39. The ethanol yield coefficient increased from 0.465 gE g^{-1}S to 0.493 gE g^{-1}S (theoretical yield is 0.54 gE g^{-1}lactose) when the feed sugar was increased from 55 g l^{-1} to 102 g l^{-1}. Further increases in the feed sugar resulted in decreases in the $Y_{P/S}$ with a yield coefficient of 0.3 gE g^{-1}S when the feed sugar was 200 g l^{-1}. The optimal feed sugar content maximizing the ethanol yield coefficient was between100 and 125 g l^{-1}. Unlike ethanol yield, the biomass yield coefficient ($Y_{X/S}$) decreased almost steadily with the increasing feed sugar content. An increase in the feed sugar content from 55 g l^{-1} to 200 g l^{-1} resulted in a decrease in the biomass yield coefficient from 0.123 gX g^{-1}S to 0.063 gX g^{-1}S.

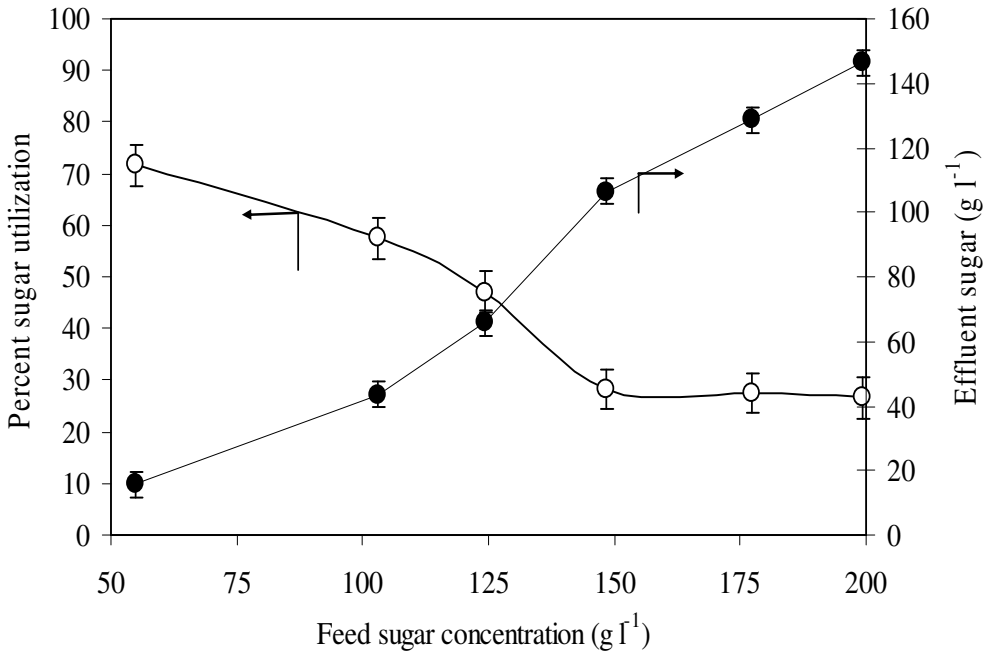


Figure 5.36 Variation of percent sugar utilization and effluent sugar content with the feed sugar concentration; V_t=3 l, HRT=54h, pH=5, ORP= -200±100 mV, 28±2 ^oC

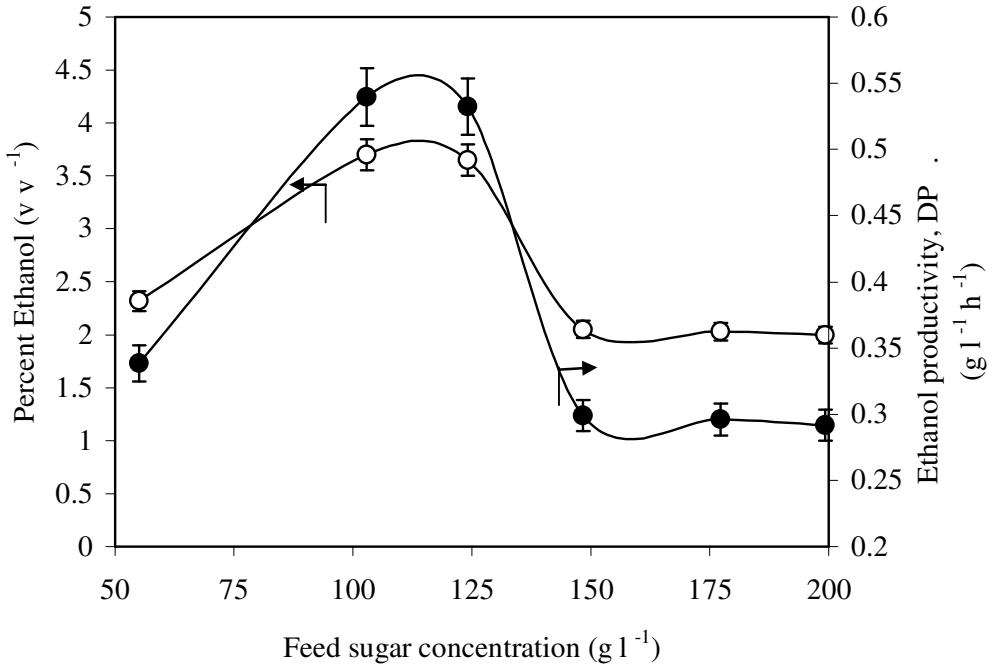


Figure 5.37 Variation of percent ethanol and ethanol productivity with the feed sugar concentration; V_t=3 l, HRT=54h, pH=5, ORP= -200±100 mV, 28±2 ^oC

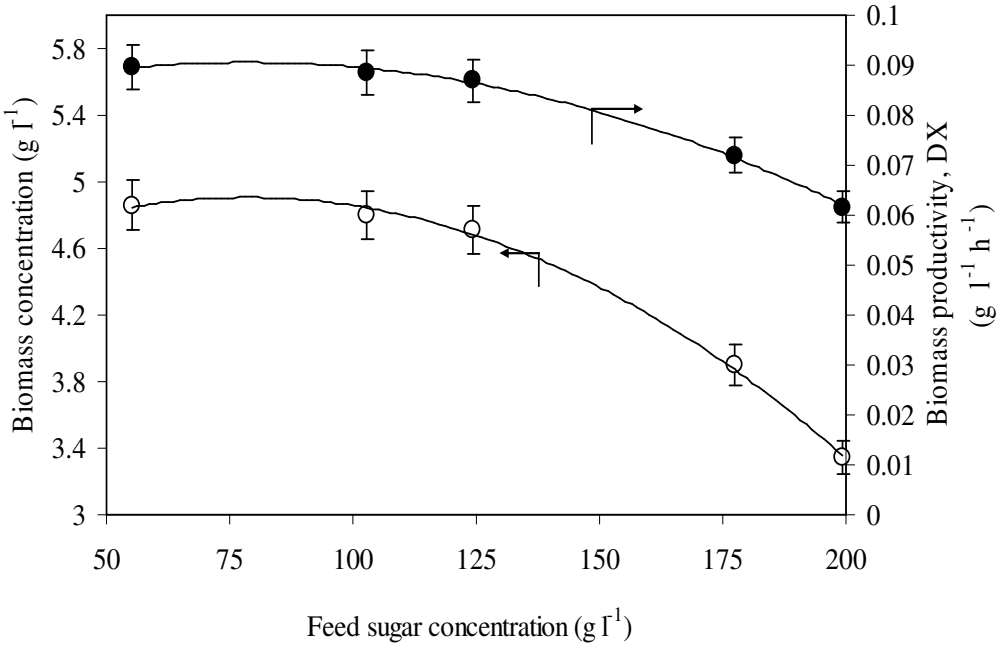


Figure 5.38 Variation of biomass concentration and biomass productivity with the feed sugar concentration; V_t=3 l, HRT=54h, pH=5, ORP= -200±100 mV, 28±2 ^oC

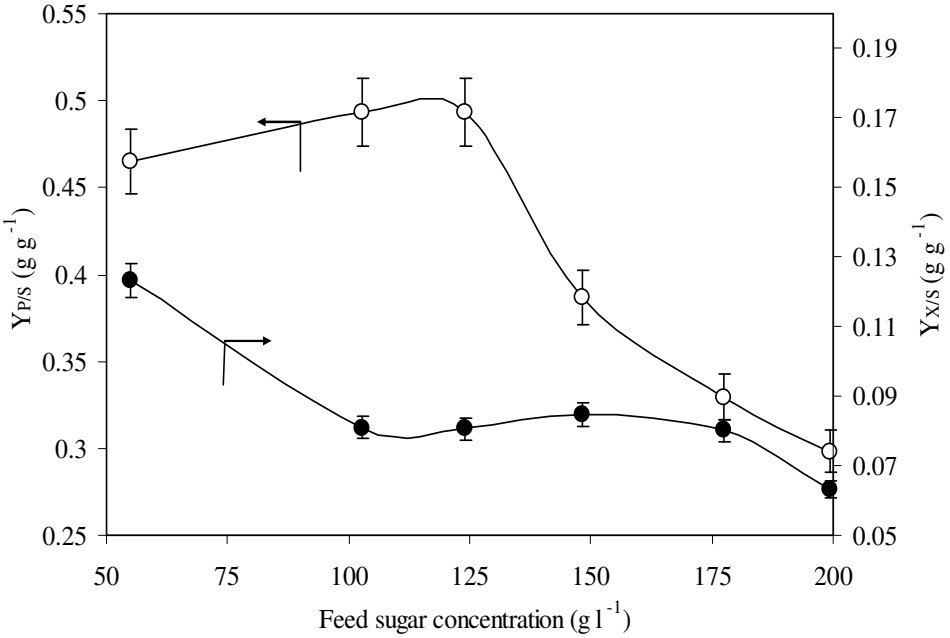


Figure 5.39 Variation of product and biomass yield coefficients with the feed sugar concentration; V_t=3 l, HRT=54h, pH=5, ORP= -200±100 mV, 28±2 ^oC

Figure 5.40 depicts variations of volumetric rates of sugar utilization and product (ethanol) formation with the feed sugar concentration where R_s and R_p were calculated by using the following equations.

$$R_S = Q\,(S_o - S) / V = D\,(S_o - S), \qquad R_P = Q\,(P - P_o) / V = D\,(P - P_o)$$

where S_o and S are the feed and effluent sugar concentrations at the steady-state (g S l^{-1}); P_o and P are the feed and effluent ethanol concentrations at the steady-state (g E l^{-1}) and P_o is zero since the feed is ethanol free; Q and V are the feed flow rate (l h^{-1}) and the volume of fermentation broth (l). Sugar utilization rate (R_s) increased with increasing feed sugar content up to 100 g l^{-1} (S_e = 44 g l^{-1}) and reached a maximum level of 1.09 gS l^{-1} h^{-1} which decreased considerably with further increases in the feed sugar above 125 g l^{-1}(S_e = 66 g l^{-1}). Ethanol formation rate showed a similar trend and increased with increasing feed sugar content up to 100 g l^{-1} and then decreased with further increases in the feed sugar above 125 g l^{-1}. The optimal feed

sugar content was between 100 and 125 g l^{-1} maximizing the rates of sugar utilization and ethanol formation.

Substrate inhibition at high sugar concentrations in ethanol fermentation has also been observed by other investigators [Ghaly and El-Taweel, 1995; 1997; Ozmihci and Kargi 2007c]. In this study, substrate inhibition was observed for the feed sugar concentrations above 125 g l^{-1} (since the results with S_o = 100 g l^{-1} and 125 g l^{-1} were not much different) corresponding to the steady-state sugar concentration in the fermenter of 66 g l^{-1}. Presence of solid cheese whey powder (CWP) and other dissolved nutrients along with sugar in the fermenter broth has also contributed to high osmotic pressure development causing inhibition on the metabolism of the yeast cells. Percent sugar utilization and ethanol formation obtained at the high feed sugar concentrations may be improved by operation with cell recycle in continuous culture.

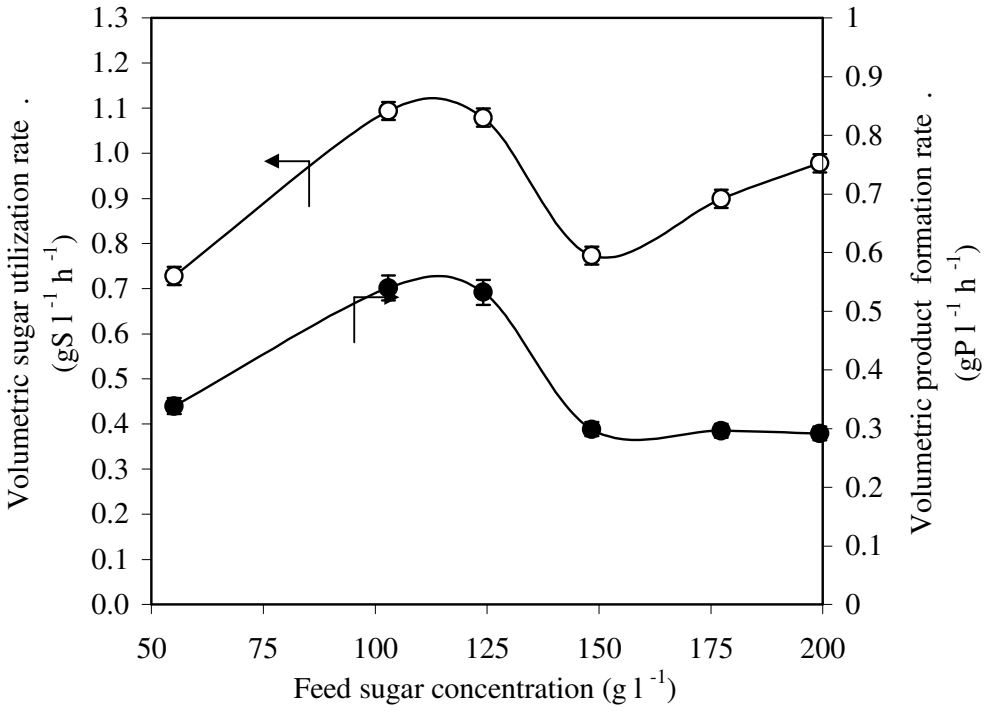


Figure 5.40 Variation of volumetric sugar utilization and product formation rates with the feed sugar concentration; V_t=3 l, HRT=54h, pH=5, ORP= -200±100 mV, 28±2 ^oC

5.4 Continuous Packed Column Biofilm Reactor (PCBR) Experiments

5.4.1 Effects of Hydraulic Residence Time

Continuous packed column experiments were performed with a constant feed sugar concentration of 50 ± 2 g l^{-1} at six different HRT's varying between 17.6 h and 64.4 h. Figure 5.41 depicts variation of ethanol concentration with the column height at different HRTs. Ethanol concentration increased with increasing column height for all HRT operations. Increase in ethanol concentrations within the first 35 cm from the inlet was rather sharp as compared to the other sections. More than 90% of the total ethanol formation took place within the 35 cm of the reactor height from the entrance port when HRT was above 25 h. This was consistent with the extensive sugar utilization within the same section of the column due to high sugar and high yeast concentration. However, at low HRTs such as 17.6 h ethanol formation and sugar utilization were more evenly distributed over the column height due to high sugar loading rates ($Q\ S_o/V$). Percent ethanol in the effluent increased with increasing HRT up to 50 h and remained almost constant for higher HRT operations. The effluent ethanol concentration increased from 10.5 g l^{-1} to 17.1 g $l^{-1}$and further to 19.8 g l^{-1} when HRT was increased from 17.6h to 37.3h and further to 50h. Effluent ethanol concentration decreased to 18 g l^{-1} at HRT = 64.4 h probably due to high maintenance requirements and low growth rates at high HRT's. Operation at HRT = 50 h yielded the highest ethanol concentration in the effluent. However, if the effluent were removed from the middle point of the reactor yielding an HRT of 15 h, the effluent ethanol would be 18 g l^{-1}.

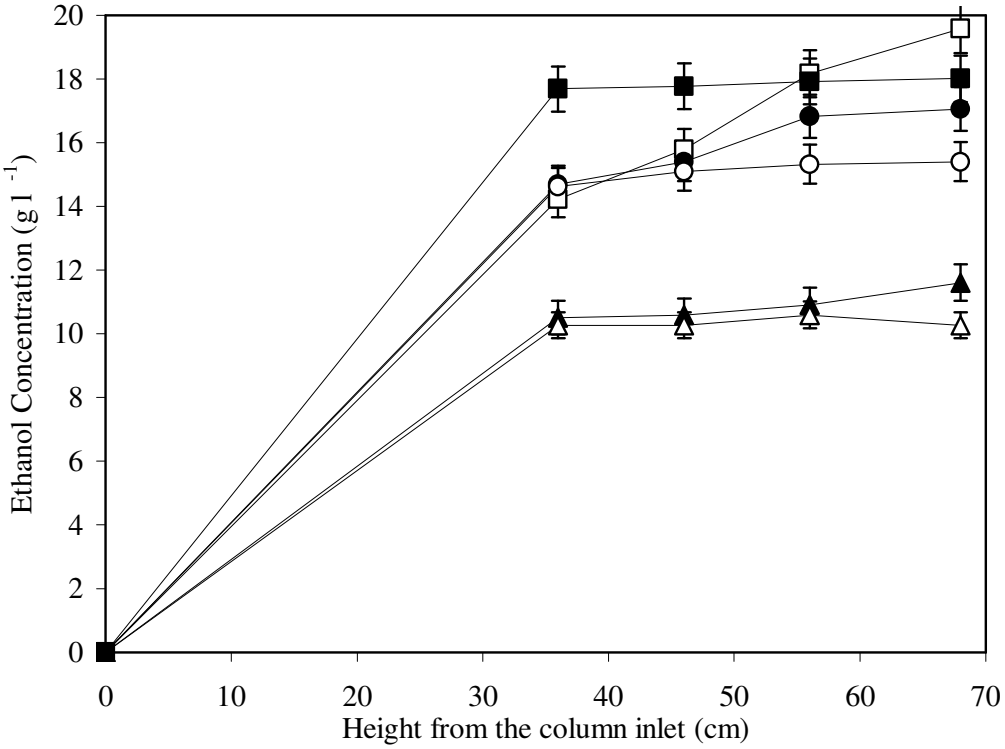


Figure 5.41 Variation of ethanol concentration with the column height for different HRT operations. HRT: (Δ) 17.6h, (▲) 22.4 h, (○) 28.4 h, (●) 37.3 h, (□) 49.8 h, (■) 64.4 h; V_t=1.79 l, So=50±2 g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^oC

Figure 5.42 depicts variations of pH and ORP with the column height for operation at HRT = 37.3 h. The feed pH was adjusted to 5.3. pH decreased from 5.3 at the inlet to nearly 4.3-4.4 and remained almost constant throughout the column. Since pH = 4.5 ± 0.2 was reported to be the optimal pH for *K. marxianus*, pH= 4.3-4.4 within the column was appropriate. The ORP was around -220mV at the inlet which remained between -225 and -250 mV throughout the column and decreased to -275 mV in the effluent. The ORP levels were also suitable sustaining anaerobic conditions throughout the column.

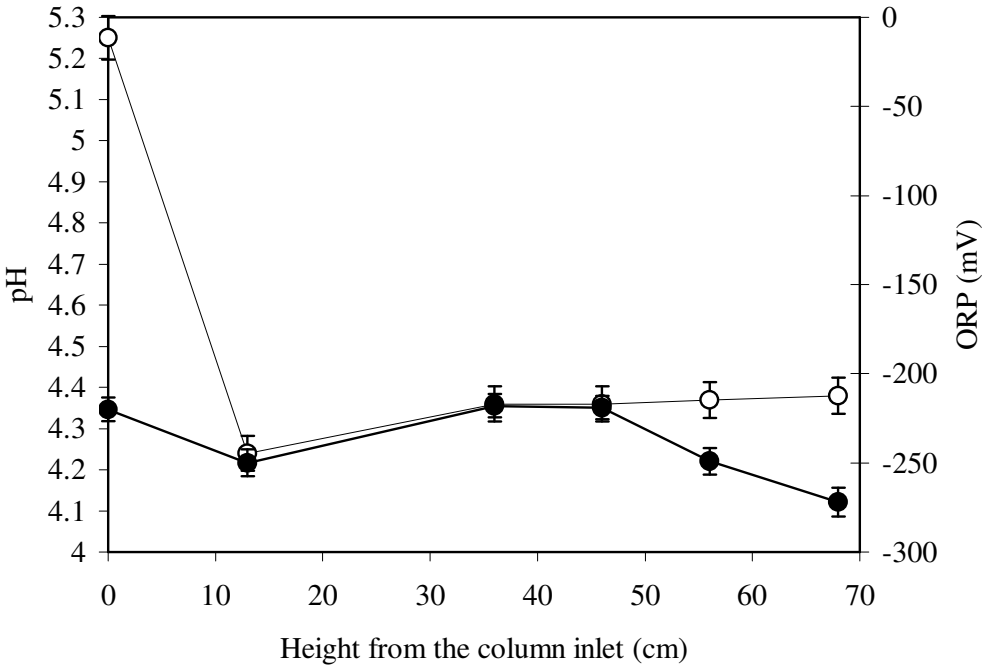


Figure 5.42Variation of pH (○) and ORP (●) with the column height for HRT 37.3 h.; V_t=1.79 l, So=50±2 g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^oC

Variations of percent sugar utilization and the effluent total sugar concentration with the HRT are depicted in Figure 5.43 for the whole column. The effluent sugar decreased and percent sugar utilization increased with increasing HRT due to longer fermentation period at high HRT operations. Percent sugar utilization increased from 63% to 68% and further to 70% when HRT increased from 17.4 h to 37.3 h and further to 50 h with effluent sugar concentrations of 19.2 g l^{-1}, 16.8 g l^{-1} and 15.3 g l^{-1}, respectively. Percent sugar utilization decreased and the effluent sugar increased slightly when HRT was 64.4 h due to high maintenance requirements and low biomass concentrations at high HRT operations. Operation at HRT = 50 h was found to be the most suitable since percent sugar utilization was maximum (70%) and the effluent sugar was minimum (15.5 g l^{-1}) at this HRT. However, if the effluent were removed from the middle of the column with an HRT of 15h (instead of 50 h) the effluent sugar would be 17 g l^{-1}. That is, the contribution of the upper section of the column was marginal and the column could be operated with one-half of the total height without much loss in sugar utilization and the ethanol formation.

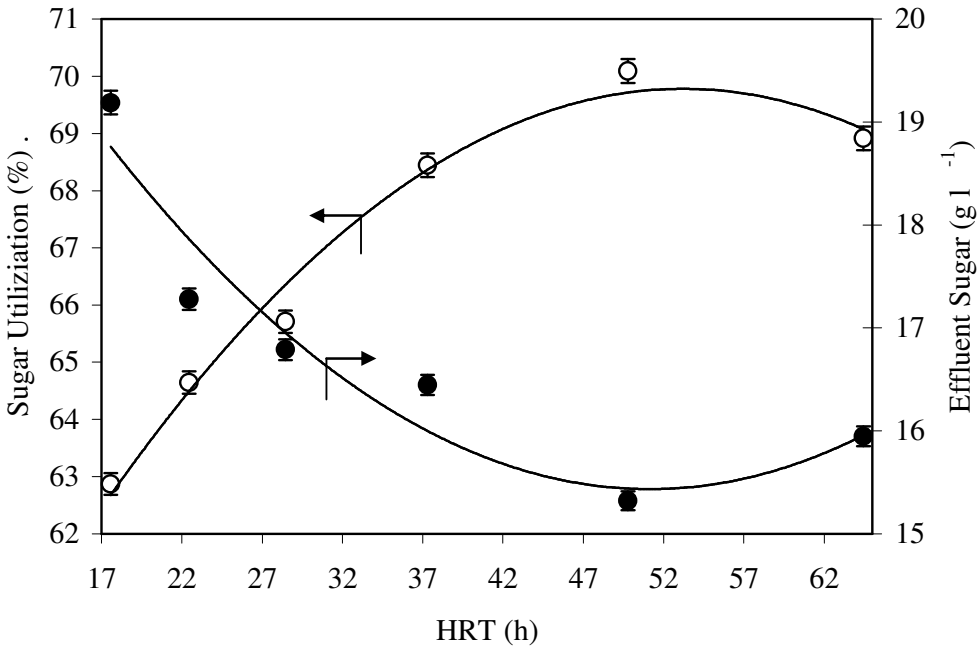


Figure 5.43 Variation of percent sugar utilization (○) and effluent sugar concentration (●) with HRT; V_t=1.79 l, So=50±2 g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^oC

Figure 5.44 depicts variations of effluent ethanol concentration and ethanol productivity (DP, gE l^{-1} h^{-1}) with the HRT for the whole column. In parallel to percent sugar utilization, effluent ethanol concentration increased with increasing HRT due to longer fermentation periods at high HRT operations. The effluent ethanol concentrations increased from 10.5 g l^{-1} to 17.1 g l^{-1} and further to 19.8 g l^{-1} when HRT was increased from 17.6 h to 37.3 h and further to 50.0 h. Further increases in HRT to 64.4 h resulted in a decrease in the effluent ethanol to 18 g l^{-1}. The optimal HRT yielding the highest effluent ethanol was 50 h based on the liquid volume in the column. Ethanol productivity (DP, gE l^{-1} h^{-1}) was maximum at the lowest HRT of 17.6 h due to the highest dilution rate of 0.057 h^{-1} despite the low effluent ethanol concentration. Ethanol productivity (DP) decreased with increasing HRT due to decreasing dilution rates (D). Ethanol productivity was nearly 0.58 g E l^{-1} h^{-1} at HRT of 17.6 h which decreased to 0.28 g E l^{-1} h^{-1} at HRT = 64.4 h. Operation at HRT = 17.6 h may maximize the ethanol productivity, but would minimize the final ethanol concentration which increases the separation costs and therefore, is not recommended.

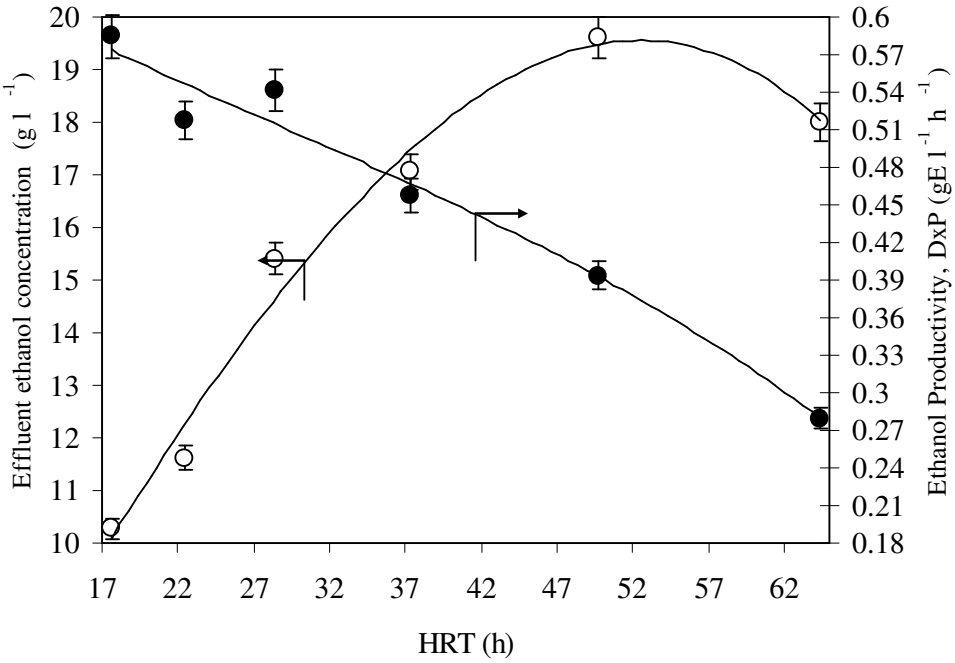


Figure 5.44 Variation of effluent ethanol concentration (○) and productivity (●) with HRT; V_t=1.79 l, So=50±2 g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^oC

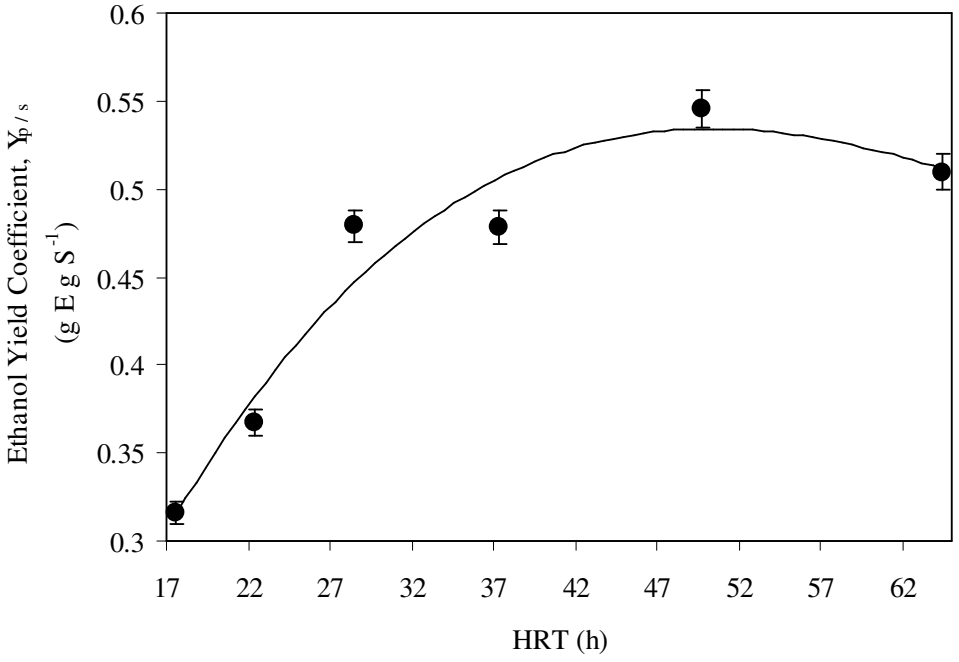


Figure 5.45 Variation of ethanol yield coefficient ($Y_{p/s}$) with HRT; V_t=1.79 l, So=50±2 g l^{-1}, pH=5, ORP= -200±100 mV, 28±2 ^oC

Figure 5.45 depicts variation of ethanol yield coefficient ($Y_{P/S}$, gE $g^{-1}S$) with HRT. The yield coefficient increased with increasing HRT up to 50 h. Further increases in HRT to 64.4 h resulted in a decrease in the ethanol yield. The lowest yield coefficient (0.32 g E $g^{-1}S$) was obtained at an HRT of 17.6 h which increased to 0.48 gE $g^{-1}S$ at HRT of 37.3 h and further to 0.54 gE $g^{-1}S$ when HTR was 50 h which is equal to the theoretical yield. The yield coefficient decreased to 0.51 gE $g^{-1}S$ when HRT was 64.4 h due to low growth rate and high maintenance requirements at high HRT operations. The optimum HRT maximizing the yield coefficient (0.54 gE $g^{-1}S$) was found to be 50 h.

The optimum HRT yielding the highest ethanol formation was found to be 50 h based on the whole liquid volume in the reactor (1.79 l). However, nearly 95% sugar utilization and ethanol formation took place within the 15 cm packed column height (i.e., 38 cm total height from the feed inlet) which is equivalent to 0.35 l liquid volume in the column. Including the 0.20 l suspended culture volume in the conical section at the bottom of the column, the total reaction volume becomes 0.55 l corresponding to an HRT of 15 h instead of 50 h. In fact the ethanol and sugar concentrations at the 15 cm column height or 38 cm reactor height from the inlet (i.e., the 2^{nd} sampling port in the column) were 19 g l^{-1} and 16 g l^{-1}, respectively which were nearly 95% the effluent concentrations. In other words, the effluent can be removed from the middle of the column instead of from the top using much lower reactor volume, but obtaining nearly the same effluent quality with an HRT of 15 h.

In our previous study (described in part 5.3.1) on ethanol fermentation from CWP in a continuously operating suspended culture reactor the optimum HRT for the highest ethanol yield was 43 h. In the PCBR used in this study the optimal HRT was found to be 15 h when the effluent was removed from the middle of the column. Due to higher biomass concentration in the reactor, utilization of PCBR is more advantageous as compared to the CSTR for ethanol fermentation from CWP solution.

5.4.2 Effects of Feed Sugar Concentration

Packed column experiments were performed at a constant HRT of 50 h based on the fermentation broth volume in the column (1.79 l) with varying feed sugar (or feed CWP) contents. An HRT of 50 h was found to be optimum maximizing the effluent ethanol content in our previous study [24]. Total sugar (TSG) content of the feed was varied between 50 and 200 g l^{-1} in order to determine the optimal feed sugar yielding the maximum ethanol content in the effluent. Figure 5.46 depicts variation of sugar concentration with the column height for different feed sugar contents. More than 90% of sugar utilization took place within the first 35 cm of the column height for all feed sugar contents. Sugar utilization in the upper section of the column was negligible due to low biomass concentration in this section. In parallel to decreasing sugar content, ethanol concentration increased with the column height as depicted in Figure 5.47 again ethanol fermentation was almost complete within the first 35 cm height of the column due to low biomass concentrations in the upper section. The highest effluent ethanol (22.5 g l^{-1}) was obtained with a feed sugar content of 100 g l^{-1}. Further increases in the feed sugar content resulted in lower ethanol contents in the effluent due to inhibitory effects (i.e., high osmotic pressure) of high sugar contents. Feed sugar content of 200 g l^{-1} resulted in the lowest effluent ethanol.

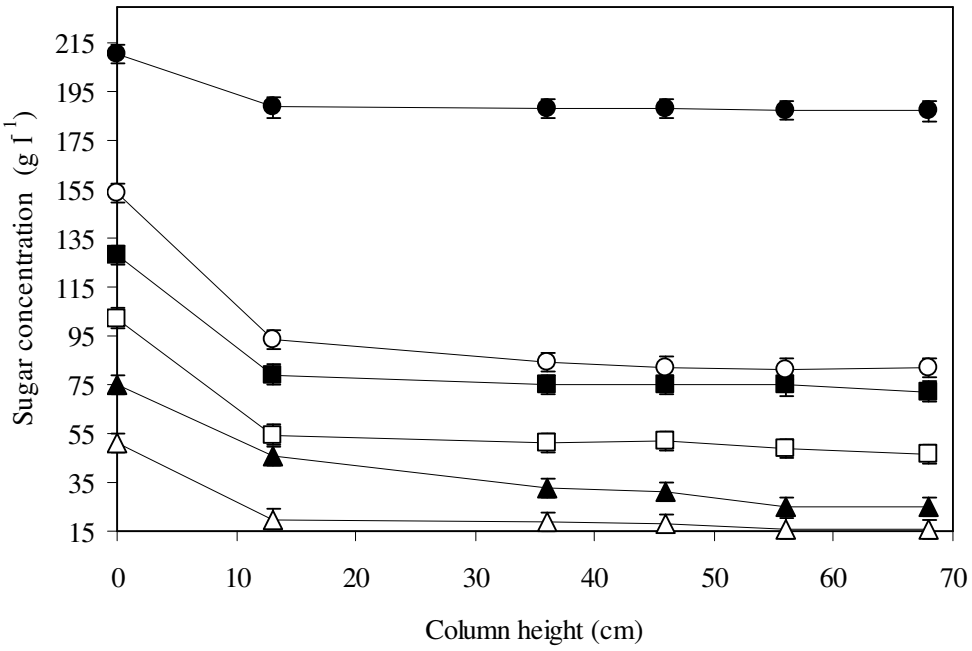


Figure 5.46 Variation of sugar concentration with the column height at different feed sugar contents (Δ) 50, (▲) 75, (□) 100, (■) 125, (○) 150, (●) 200 g l^{-1}; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

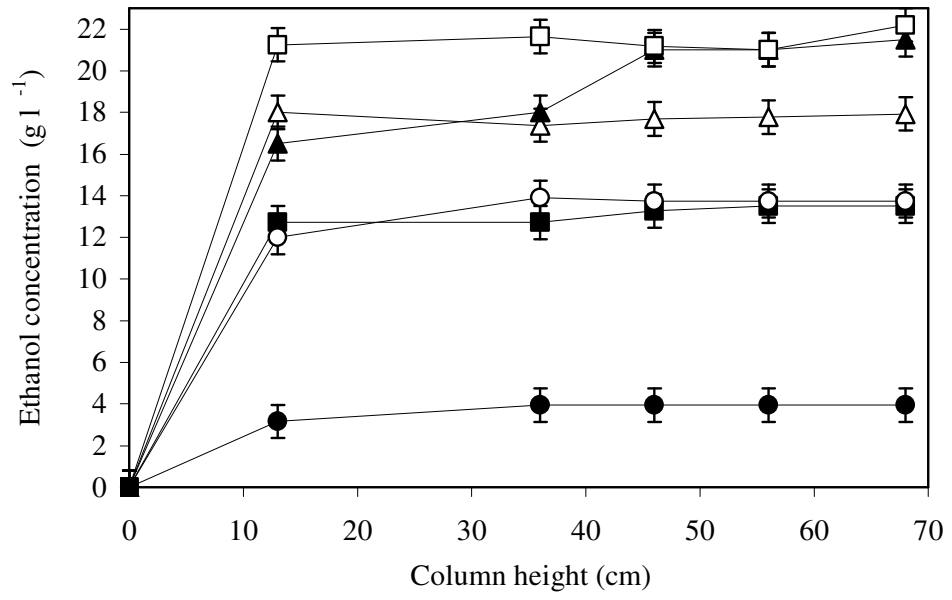


Figure 5.47 Variation of ethanol concentration with the column height at different feed sugar contents. (Δ) 50, (▲) 75, (□) 100, (■) 125, (○) 150, (●) 200 g l^{-1}; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

Figure 5.48 depicts variation of suspended biomass concentration (X_s, g l^{-1}) with the column height. The biomass concentration decreased with the column height for all feed sugar contents not necessarily due to unavailability of sugar in the upper sections of the column, but probably due to sedimentation of he yeast cells at low flow rates. About 60% of the total biomass was in the suspended form and 40% was attached on the particle surfaces. Therefore, the suspended cells settled at the bottom of the column yielding low cell concentrations in the upper section although high sugar contents were available in the upper section of the column. Biomass settling is the major reason for low cell concentrations and therefore, low sugar utilization and low ethanol formation in the upper section of the column.

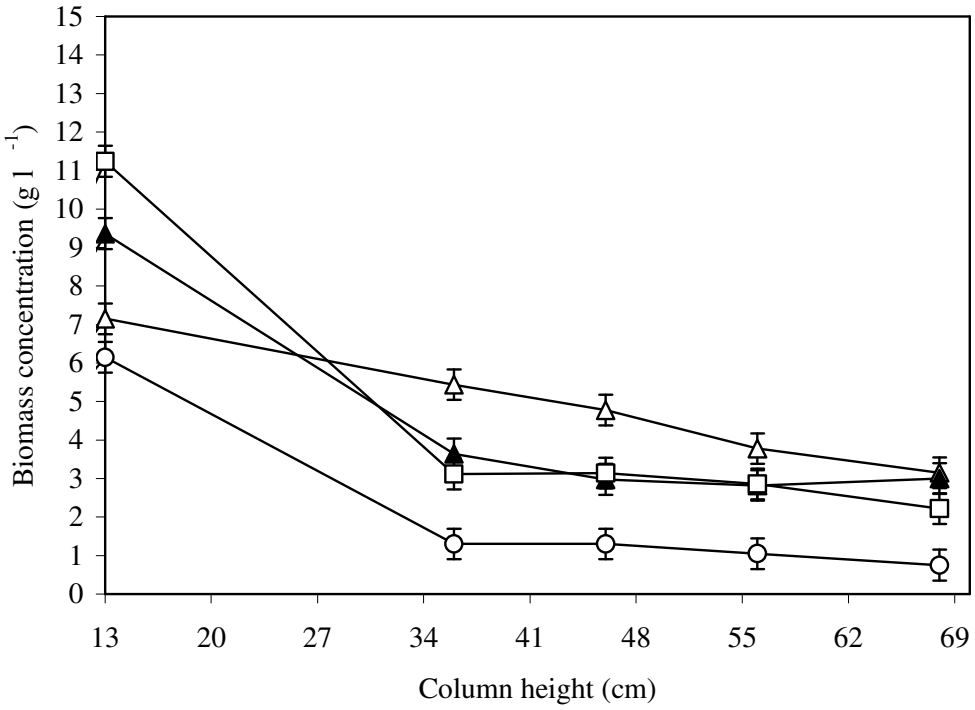


Figure 5.48 Variation of suspended biomass concentration with the column height at different feed sugar contents. (Δ) 50, (▲) 75, (□) 100, (○) 150 g l^{-1}; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

Variation of effluent sugar content and percent sugar utilization with the feed sugar content are depicted in Figure 5.49. Percent sugar utilization between the inlet

and the outlet of the column decreased with increasing feed sugar content due to cell inactivation by high osmotic pressure at high sugar contents. The highest percent sugar utilization (72%) was obtained with the lowest feed sugar of 50 g l^{-1} which decreased to nearly 15% with a feed sugar of 200 g l^{-1}. In parallel to percent sugar utilization, the effluent sugar contents increased with increasing feed sugar content yielding the lowest effluent sugar (15 g l^{-1}) for a feed sugar of 50 g l^{-1}.

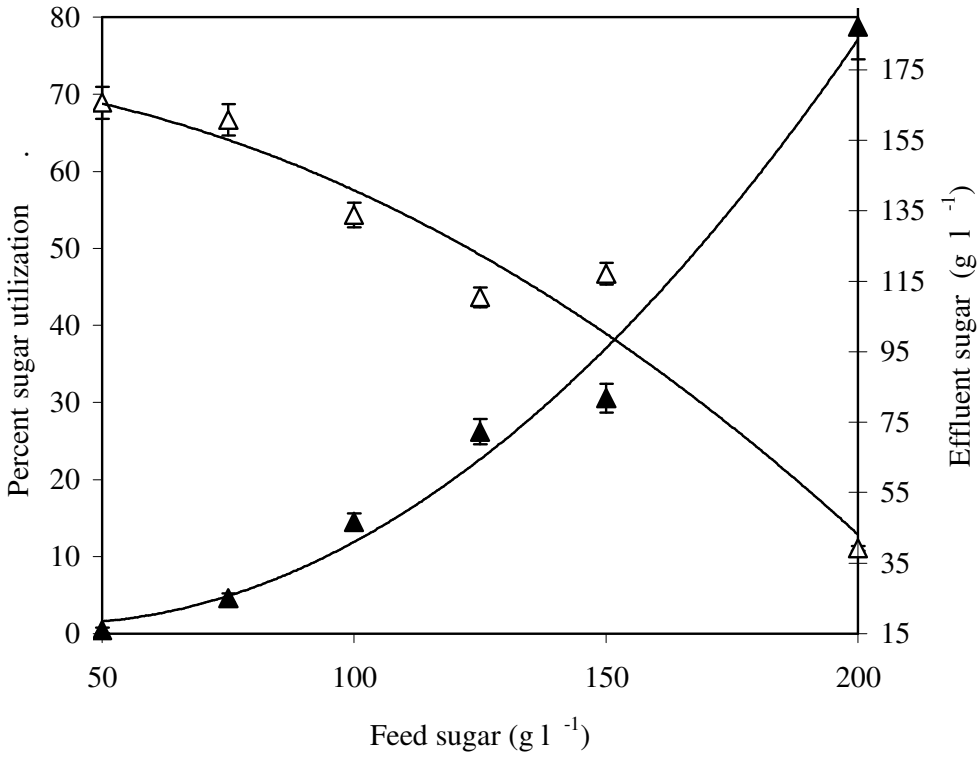


Figure 5.49 Variation of percent sugar utilization (Δ) and effluent sugar concentration (▲) with the feed sugar content; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 °C

Figure 5.50 depicts variation of the effluent ethanol contents with the feed sugar content. Effluent ethanol increased with increasing feed sugar up to 100 g l^{-1} and reached the maximum level of 22.5 gEtOH l^{-1}. Further increases in the feed sugar resulted in decreases in effluent ethanol due to lower levels of sugar utilization. Low feed sugar contents (< 100 g l^{-1}) caused substrate limitations while high sugar contents (> 100 g l^{-1}) resulted in substrate inhibition due to high osmotic pressure.

The system should be operated with a feed sugar content of 100 g l^{-1} to obtain the highest effluent ethanol.

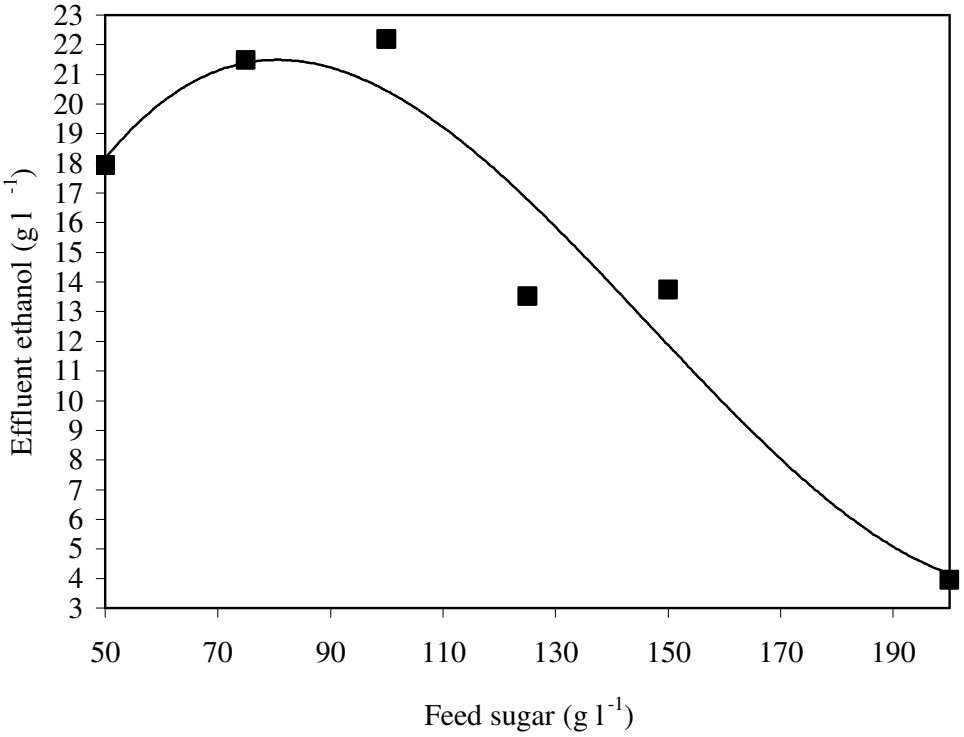


Figure 5.50 Variation of effluent ethanol concentration with the feed sugar content; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

The ethanol yield coefficient ($Y_{p/s}$) also varied with the available sugar or the feed sugar content since the yeast metabolism was regulated by the available sugar. Variation of the ethanol yield coefficient with the feed sugar content is depicted in Figure 5.51. The yield coefficient decreased with increasing feed sugar due to adverse effects of high sugar contents. The maximum yield (0.52 gE g^{-1}S) was obtained with a feed sugar content of 50 g l^{-1} which is almost equal to the theoretical yield coefficient (0.54 gE g^{-1}lactose). High sugar contents had adverse effects on ethanol formation and also might have inactivated the cells due to high osmotic pressure encountered at high sugar contents causing high maintenance requirement

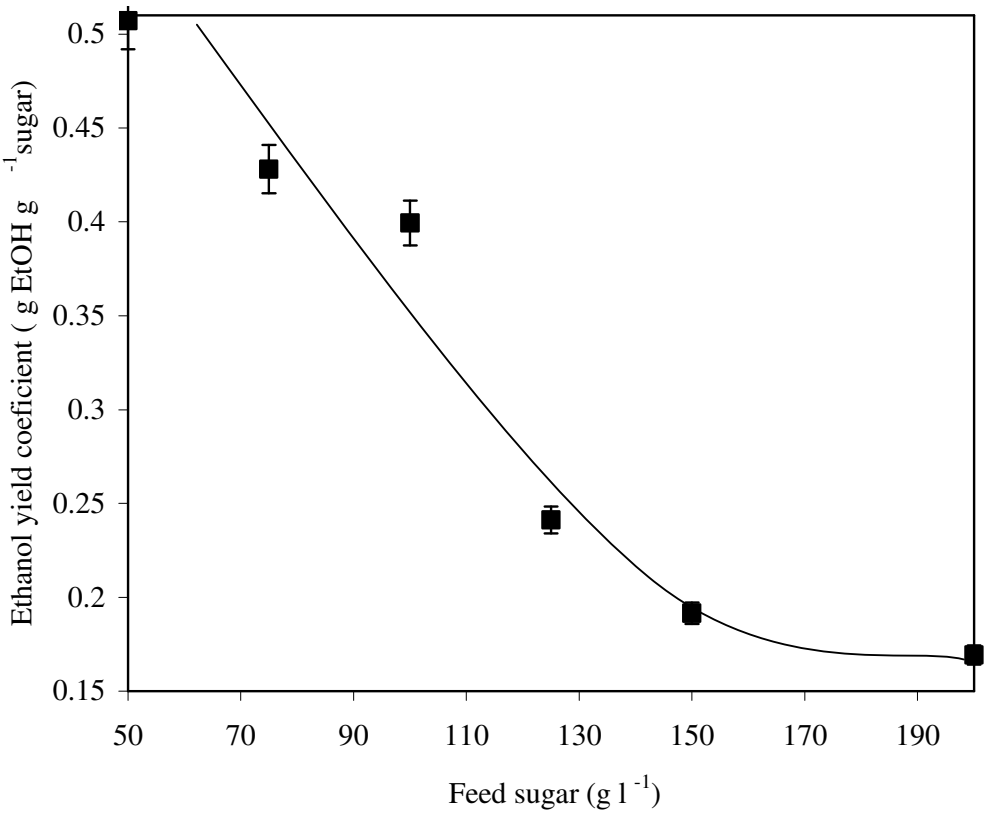


Figure 5.51 Variation of ethanol yield coefficient with the feed sugar content; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

The data presented in Figure 5.47 was used to determine the specific rate of ethanol formation (q_p) for different feed sugar contents. Variation of ethanol concentration with the column height was not significant for the column heights above 35 cm and was the most significant within the first 13 cm of the column. Eqn 14 can be rewritten as follows

$$q_p = \frac{P - P_o}{\theta_H X} = \frac{\Delta P}{(A_o Z/Q) X} \qquad \text{(Eqn 16)}$$

The difference in ethanol concentrations (ΔP) within the first 35 cm column height (Z = 0.35 m), Q = 0.036 l h^{-1}, V =0.51 l, θ_H = 14.2 h and the average biomass concentration within this section of the column (X, g l^{-1}) were used to calculate the q_p values for every feed sugar concentration using eqn 16. The q_p values were plotted versus the feed sugar content in Figure 5.52. The specific rate of ethanol formation (q_p) increased with increasing feed sugar and reached the maximum level at 100 g l^{-1} feed sugar content. Further increases in the feed sugar above 100 g l^{-1} resulted in decreases in the q_p due to adverse effects of high sugar contents causing high osmotic pressures and therefore, high maintenance requirements. The optimum feed sugar content maximizing the specific rate of ethanol formation was 100 g l^{-1}.

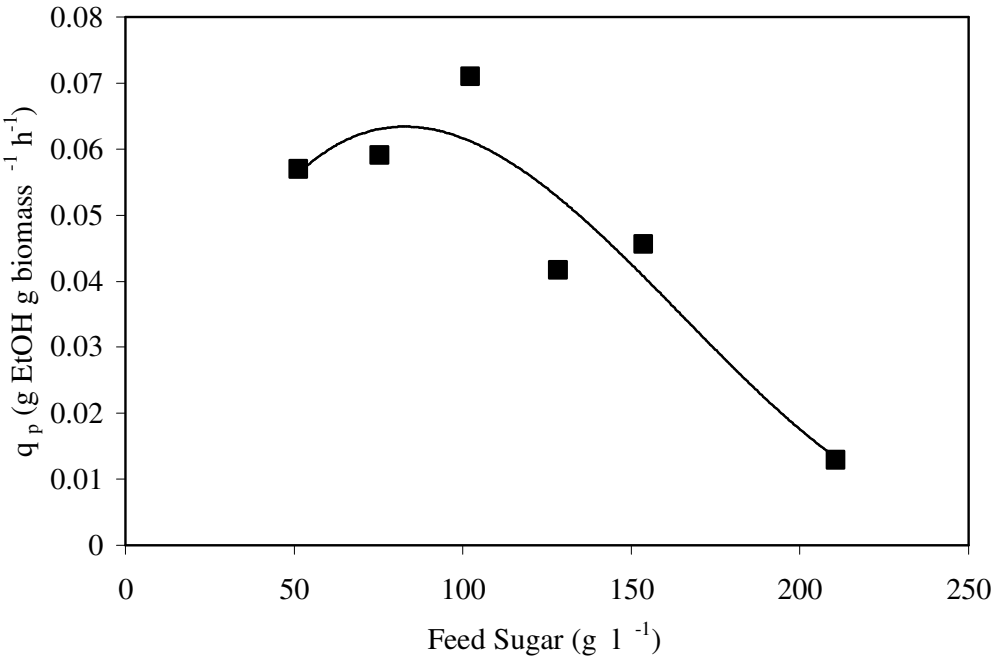


Figure 5.52 Variation of the specific rate of ethanol formation with the feed sugar content; V_t=1.79 l, HRT= 50 h, pH=5, ORP= -200±100 mV, 28±2 ^oC

5.5 Comparison of the Ethanol Production Systems

Ethanol production from cheese whey powder (CWP) solution was investigated using batch, fed-batch and continuous fermentation systems. The operational conditions and the best results for different methods are summarized inTable .2

Batch fermentations are difficult to operate at high initial sugar contents due to substrate inhibition. Batch fermentation is a dynamic system with variable effluent quality and also takes a long time with lower ethanol productivity. Continuous operation provides constant product quality at the steady-state. However, the effluent ethanol concentration is determined by the HRT at a constant feed sugar content. In continuous suspension culture operation, the optimum HRT was 43.2 h with ethanol concentration and productivity of 42 g l^{-1} and 0.97 g EtOH l^{-1} h^{-1}, respectively. The highest ethanol productivity (0.57 g EtOH l^{-1} h^{-1}) in batch operation was obtained with the initial sugar concentration of 100 g l^{-1}. Continuous operation was found to be preferable over batch operation due to higher ethanol productivities.

Repeated fed-batch operation is used at high feed sugar contents in order to overcome substrate inhibition. In repeated-fed batch operation up to 8% ethanol concentrations were obtained at the end of the fifth cycle yielding 63 g l^{-1} ethanol concentration. The highest ethanol productivity in fed-batch operation was 1.31 g EtOH l^{-1} h^{-1} (obtained with the feed sugar content of 125 g l^{-1}) which was considerably higher than those of the batch and continuous operations.

Biomass concentration in the PCBR system was above 5 g l^{-1} yielding high rates of ethanol fermentation. The lowest HRT obtained with the PCBR was 15 h with a feed WP of 100 g l^{-1} yielding an effluent ethanol content of 22.5 g l^{-1}. Ethanol productivity under these conditions was 1.50 g EtOH l^{-1} h^{-1} which is superior to other operations.

On the basis of the ethanol productivities, the PCBR is preferable over the other suspension culture operations due to high biomass concentrations yielding high ethanol productivities. Fed-batch operation is the best choice among the suspended culture operations yielding higher ethanol productivities.

Table 5.2 Operational conditions for different methods used for fermentation of ethanol from cheese whey powder.

System	Sugar concentration ($g\ l^{-1}$)	Agitation	pH	ORP (mV)	Feed flow rate ($ml\ h^{-1}$)	Retention time	Ethanol concentration ($g\ l^{-1}$)	$Y_{P/S}$ ($g\ EtOH\ g\ S^{-1}$)	$Y_{X/S}$ ($g\ X\ g\ S^{-1}$)	Bimass concentration ($g\ l^{-1}$)	Productivity ($g\ EtOH\ l^{-1}\ h^{-1}$)
Batch	100	150 rpm	5 - 4.6			72 h	41.08	0.54		min. 8.50	0.57
Fed-Batch	125	100 rpm	4.7-4.2	-250±50	84	48 h (5 cycle)	63	0.475	0.1	~7.50	1.31
Continuous Fermentor											
Var. HRT	100	100 rpm	4.5	-250±50	70	43.2 h	42	0.4	0.1	8.0	0.97
Var. CWP	100-125	100 rpm	4.5	-250±50	56	54 h	29.23	0.49	0.12-0.6	5.6	0.54
Continuous PCBR											
Var. HRT	50		4.3-4.6	-250±50	36	15 h	19	0.54		5.0	1.27
Var. CWP	100		4.2-4.5	-250±50	36	15 h	22.5	0.41		11.50-3.00	1.50

There is no literature reports on ethanol production from cheese whey powder solution except our studies. However, whey and ultrafiltrated whey was used for ethanol fermentation. The highest ethanol concentration in batch fermentations was obtained in our studies (42 g l^{-1}). In literature reports on batch ethanol fermentations, ethanol concentration varied between 2 and 30 g l^{-1}. (Grba et al., 2002; Longhi et.al, 2004; G. Cortes, 2005; Zafar S & Owais M., 2006; Lukondeh T. et. al. 2005). Higher ethanol concentrations (60 g l^{-1}) were obtained from whey permeate using fed-batch operation(Grba et al., 2002) which is also lower then our results (63 g l^{-1}). Ethanol productivities obtained in our study are comparable with the literature reports (Belem & Lee, 1998; Lukondeh T. et. al. 2005; Altıntaş et.al. 2002) Using cheese whey powder instead of cheese whey improved ethanol fermentation yielding high ethanol productivities. Ethanol production can further be improved by using continuous operation with cell recycle.

CHAPTER SIX
CONCLUSIONS

At the beginning of this study, three different substrate cheese whey (CW), cheese whey powder (CWP) and lactose; and two different *Kluyveromyces marxianus* strains (NRRL-1109, NRRL-1195) were used to find out the most suitable substrate with the highest ethanol yield. The most suitable media was found to be cheese whey powder (CWP) which was a concentrated form of cheese whey and can be used for ethanol fermentations in desired concentrations. *K marxianus*- NRRL-1195 performed better than the NRRL-1109 in ethanol fermentation of CWP solution.

The effects of initial pH, external nutrient addition and CWP concentrations on ethanol formation rate and extent were also investigated in batch fermentation. A *Kluyveromyces marxianus* strain of NRRL-1195 was used for this purpose. The most suitable initial pH was found to be 5 resulting in maximum final ethanol concentration and ethanol formation rate. External addition of N and P sources to the CWP solution did not improve ethanol formation and sugar utilization indicating the fact that CWP was well balanced in terms of N and P contents for ethanol fermentation. Ethanol formation from CWP solution was also realized with different CWP or sugar concentrations between 52 and 312 g CWP l^{-1} or 26 and 156 g sugar l^{-1}. High initial sugar concentrations above 100 g l^{-1} resulted in low fermentation rates due to substrate inhibition. However, the final ethanol yield and ethanol formation rate increased with CWP and sugar concentration indicating no substrate and product inhibitions, but possible substrate limitations within the range of sugar concentration tested.

In later stages of batch experiments ethanol formation from CWP solution was investigated using two different strains of *K. marxianus* NRRL-1195 and DSMZ-7239. Both sugar utilization and ethanol formation performance of DSMZ 7239 was better than NRRL- 1195. Therefore, *K.marxianus*-DSMZ 7239 was used in further experiments. Ethanol formation from cheese whey powder (CWP) solution was investigated as functions of pH, ORP, the substrate (CWP) and biomass concentrations in batch shake flask experiments using the *K.marxianus* DSMZ-7239.

Initial pH of 5 and initial Na-thioglycolate concentration of 200 mg l^{-1} was found to be the most suitable.

Ethanol formation from cheese whey powder (CWP) solution was investigated as functions of the substrate (CWP) and biomass concentrations using batch experiments with *Kluyveromyces marxianus* DSMZ-7239. The rate and extent of ethanol formation or sugar utilization increased with increasing CWP or sugar concentration up to 156 g l^{-1} CWP (78 g l^{-1} sugar) concentration indicating substrate limitation at low CWP or sugar concentrations. Further increases in CWP concentration above 156 g l^{-1} resulted in gradual decreases in the rate and extent of ethanol formation indicating substrate inhibition at high CWP or sugar concentrations. The ethanol yield coefficient was also equal to the theoretical yield (0.54 g E g S^{-1}) for CWP concentrations below 156 g l^{-1}, which decreased to nearly 0.25 gE. gS^{-1} at CWP concentration of 312 g l^{-1}. CWP concentrations should be kept below 156 g l^{-1} (sugar < 78 g l^{-1}) in batch fermentations to avoid substrate inhibition possibly due to high osmotic pressure. Fed-batch fermentations may also be used to overcome substrate inhibition at high CWP or sugar concentrations. Increasing biomass concentrations resulted in improved sugar utilization and ethanol formation. Both the rate and the extent of ethanol formation increased almost linearly with the biomass concentrations between 170 and 1020 mg l^{-1}. Maximum ethanol concentration of 3.65% (v v^{-1}) was obtained with 1020 mg l^{-1} biomass concentration. The yield coefficient ($Y_{P/S}$) also increased with biomass concentration and reached the theoretical value when initial biomass was 1020 mg l^{-1}. A high biomass concentration above 510 mg l^{-1} was advantageous resulting in shorter fermentation times and higher yield and extent of ethanol formation.

In order to overcome substrate inhibition at high CWP concentrations in batch operation, repeated-fed-batch operation was used with slow addition of CWP solution. Feed sugar concentration was varied between 25 and 200 g l^{-1} and *Kluyveromyces marxianus* (DSMZ 7239) was used in five-cycle repeated fed-batch operation. Sugar utilization, ethanol formation and the yeast growth were quantified while the feed flow rate (0.084 l h^{-1}) and the other environmental conditions were constant. The system reached quasi-steady state at the end of the fifth-cycle resulting

in constant sugar, ethanol and biomass concentrations. Percent sugar utilization decreased with increasing feed sugar concentration while percent ethanol concentration was maximum with a feed sugar content of 125 g l^{-1}. The growth yield coefficient ($Y_{x/s}$) also decreased with increasing feed sugar content due to high osmotic pressure at high sugar concentrations. The maximum ethanol yield coefficient ($Y_{p/s}$) was obtained at a feed sugar content of 125 g l^{-1}. Ethanol productivity also increased with the increasing sugar loading rate up to 10.5 g sugar h^{-1} and then decreased due to substrate inhibition at high sugar loading rates. The highest ethanol concentration (63 g l^{-1}) and the productivity 5.3 g EtOH h^{-1} was obtained with 125 g l^{-1} feed sugar concentration (Ls =10.5 g S h^{-1}). The biomass yield coefficient decreased with increases in the feed sugar concentration. The highest ethanol concentration (63 g l^{-1}) and the productivity (0.91 g E l^{-1} h^{-1}) was obtained with 125 g l^{-1} feed sugar concentration (L_r=1.8 g S l^{-1} h^{-1}). At high feed sugar concentrations above 125 g l^{-1}, high osmotic pressure and product inhibition adversely affected the system. The highest ethanol yield coefficient (0.475 g g^{-1}) was also obtained with 125 g l^{-1} initial sugar concentration.

Ethanol fermentation of CWP solution was also investigated by continuous operation. Cheese whey powder solution with sugar concentration of 100 ± 5 g l^{-1} was fermented to ethanol using *Kluyveromyces marxianus* (DSMZ 7239) in a continuous fermenter under anaerobic conditions at different HRT levels of between 12.5 and 60 hours. The pH, temperature and the ORP in the fermenter were around 4.5, 28 ^{o}C and -250 mV, respectively. Sugar utilization, ethanol formation and the yeast growth were quantified as function of HRT and the yield coefficients were determined as well as the optimal operating HRT. The steady-state effluent sugar concentrations decreased, but ethanol and biomass concentrations increased with HRT due to higher sugar utilizations at high HRT levels. Ethanol productivity (DP) was maximum (0.745 gE l^{-1} h^{-1}) at an HRT of 43.2 h where the biomass productivity (DX) was almost minimum (0.18 gX l^{-1} h^{-1}). The ethanol yield coefficient ($Y_{P/S}$) was almost constant at 0.4 gE g^{-1}S up to HRT of 43.2 h which increased to 0.496 gE g^{-1}S at an HRT of 60 h. The growth yield coefficient was minimum at HRT of 43.2 h yielding the lowest biomass productivity. The system should be operated at an HRT of 43 h in order to maximize the ethanol and to minimize the biomass productivities.

The maximum growth yield coefficient was found to be Y_M = 0.20 gS g^{-1}X. The basal metabolism rate constant (b) was negligible.

As compared to the literature reports on cheese whey fermentations, the maximum ethanol productivity obtained in this study is better than most of the related studies due to high sugar concentrations in the feed. Ethanol productivity can be further improved by using more concentrated CWP solution with higher sugar contents.

Continuous fermentation of cheese whey powder (CWP) solution to ethanol was also investigated at different feed sugar concentrations (55-200 g l^{-1}). *Kluyveromyces marxianus* (DSMZ 7239) was used in a continuous fermenter under anaerobic conditions at HRT = 43 h. Sugar utilization, ethanol formation and the yeast growth were quantified at different feed sugar concentrations varying between 55 and 200 g l^{-1}. The steady-state effluent sugar concentration increased and percent sugar removal decreased with increasing feed sugar content due to high osmotic pressure caused by high sugar concentrations. Ethanol concentration (P) and productivity (DP) was maximum (3.7% vv^{-1}, and 0.54 gE $l^{-1}h^{-1}$) at the feed sugar concentration of 100 g l^{-1} which decreased with further increases in the feed sugar. Steady-state biomass concentration (X) and productivity (DX) also decreased considerably for the feed sugar contents above 100 g l^{-1} indicating adverse effects of high sugar contents on the yeast growth. The ethanol yield coefficient ($Y_{P/S}$) was also maximum at the feed sugar content of 100 g l^{-1} and decreased with further increases in the sugar content above 125 g l^{-1}. Biomass yield coefficient decreased steadily with the increasing feed sugar concentration where the decrease was more pronounced at sugar concentrations above 100 g l^{-1}. Similar to the other results, the rate of sugar utilization and ethanol formation was also maximum when the feed sugar content was 100 g l^{-1}. The results obtained with 125 g l^{-1} feed sugar content were not much different from those obtained at 100 g l^{-1} and considerable decreases were observed above 125 g l^{-1} feed sugar. Therefore, the optimal feed sugar content was between 100 and 125 g l^{-1} maximizing the rate and extent of ethanol formation from the CWP solution.

All batch, fed-batch and continuous experiments were done with suspended culture. Biofilm cultures provide higher biomass concentrations and therefore faster

fermentation rates and smaller reactor volumes as compared to suspendd cultures. For this reason, a packed column biofilm reactor (PCBR) operating in up-flow mode was used for ethanol production from CWP solution containing 50 g l^{-1} total sugar at different HRTs. Percent sugar utilization and effluent ethanol concentrations increased with increasing HRT. Nearly 70% sugar utilization and 19.5 g l^{-1} ethanol concentrations were obtained in the effluent at an HRT of 50 h based on the total liquid volume in the system. Further increases in HRT to 64.4 h resulted in a decrease in the effluent ethanol concentration to18 g l^{-1}. The ethanol yield coefficient ($Y_{P/S}$) also increased with increasing HRT and reached the highest level (0.54 gE g^{-1}S) at HRT of 50 h. Sugar concentrations decreased and the ethanol contents increased with the column height due increasing fermentation time with the column height. Nearly 95% of the sugar utilization and ethanol formation took place within the first 35 cm from the reactor inlet due to availability of high sugar contents and formation of high biomass within this region. Therefore, a packed column with a height of 15 cm or HRT of 15 h would be sufficient for high sugar utilization (70%) and ethanol yields (19 g l^{-1}). The PCBR was found to be a compact and effective reactor for ethanol production from CWP solution with high ethanol yields as compared to the continuous suspended cell bioreactors.

Effects of feed sugar content on ethanol formation was also investigated in the PCBR using different feed sugar contents between 50 and 200 g l^{-1} while the HRT was constant at 50 h. Total sugar concentration decreased with increasing ethanol concentrations along the column height. Biomass concentration also decreased with the column height due to sedimentation of suspended biomass at low flow rates. Therefore, most of the sugar utilization and ethanol formation took place within the first half of the column. The highest effluent ethanol concentration (22.5 g l^{-1}) was obtained with a feed sugar content of 100 g l^{-1}. The ethanol yield coefficient ($Y_{p/s}$) decreased with increasing feed sugar content due to high maintenance requirements at high feed sugar contents. Operation with a column height of 35 cm was found to be satisfactory since not much ethanol formation was observed in the upper sections of the column due to low biomass concentration.

Recommendations for future studies:

Some recommendations for the future studies are listed bellow:

- Ethanol production from CWP solution can be investigated using different reactor types such as immobilized cells and hybrid reactors.
- Other lactose fermenting yeast cultures may be used in form of pure or mixed cultures for fermentation of CWP solution.
- Simultaneuous ethanol formation and separation can be investigated to overcome product inhibition.
- More economical ethanol separation methods, instead of distillation, can be developed.
- Ethanol formation from CWP solution can be investigated in pilot scale with ethanol separation
- High temperature (50-60 ^{o}C) ethanol fermentation processes can be developed to improve simultaneous ethanol separation.
- Economic feasibility of ethanol production from CWP can be investigated and compared with the different alternatives

REFERENCES

Agu, R.C., Amadife, A.E., Ude, C.M., Onyia, A., Ogu, E.O., Okafor, M., & Ezejiofor, E., (1997). Combined heat treatment and acid hydrolysis of cassava grate waste (CGW) biomass for ethanol production. *Waste Manag*. 17, 91-96.

Addison K. (2008). the benefits.Retrieved December 17, 2008, from http://journeytoforever.org/ethanol.html

Aksu, Z., (2005). Application of biosorption for the removal of organic pollutants: a review, *Process Biochem*. 40, 997-1026.

Altıntaş M. M., Ülgen K. Ö., Kırdar B., Önsan Z. I., & Oliver S. G. (2002). Improvement of ethanol production from starch by recombinant yeast through manipulation of environmental factors, *Enzyme and microbial technology* 31, 640-647

Bailey, J. E.; & Ollis D. F. (1986). *Biochemical Engineering Fundamentals*. 2nd edition. McGraw Hill, USA,.

Banat, I.M., Nigam, P., & Marchant, R., (1992). Isolation of thermotolerant fermentative yeast capable of growth at 52 ^{o}C and ethanol production at 45 ^{o}C and 50 ^{o}C. *World. J. Microb. Biotechnol*. 8, 259-263.

Banat, I.M., & Marchant, R., (1995). Characterization and potential industrial applications of five novel, thermotolerant, fermentative yeast strains. *World. J. Microb. Biotechnol*. 11: 304-306.

Banat, I.M., Singh, D., & Marchant, R., (1996). The use of a thermotolerant fermentative *Kluyveromyces marxianus* IMB 3 yeast strain for ethanol production. *Acta Biotechnol*. 16, 215-223.

Barba D., Beolchini F., Del Re G., Giacomo G. D., & Veglio F. (2001). Kinetic analysis of Kluyveromyces lactic fermentation o whey: batch and fed- batch operations, *Process Biochemistry* 36, 531-536

Brady, D., Nigam, P., Marchant, R., & McHale, A.P., (1997). Ethanol production at 45 ^{o}C by alginate-immobilized *Kluyveromyces marxianus* IMB3 during growth on lactose-containing media. *Bioprocess Eng*. 16, 101-104.

Brown L. R, (2008). Why ethanol production will drive world food prices even higher in 200, *China Green Companies Program* 2008, January 25, 2008.

Bruggen B. V., Geens J., & Vandecasteele C. (2002). Fluxes and rejections for nanofiltration with solvent stable polymeric membranes in water, ethanol and n-hexane, *Chemical Engineering Science* 57, 2511 – 2518

Buyanov A. L., Revel'Skaya L. G., Kuznetzov Y. P., & Khrıpunov A.K. (2001). Cellulose–Poly(acrylamide–acrylic acid) Interpenetrating Polymer Network Membranes for the Pervaporation of Water–Ethanol Mixtures. II. Effect of Ionic Group Contents and Cellulose Matrix Modification, *Journal of Applied Polymer Science*, Vol. 80, 1452–1460

Chen, H.C.,& Zall, R. R., (1982). Continuous fermentation of whey into alcohol using an attached film expanded-bed reactor. *Process Biochem.* Jan-Feb, 20-25.

Cheryan, M., & Mehaia, M.A., (1983). Ahigh performance membrane bioreactor for continuous fermentation of lactose to ethanol. *Biotechnol. Lett*. 5, 519-522

Cheung, A.W., & Anderson B.C., (1997). Laboratory investigation of ethanol production from municipal primary wastewater solids. *Bioresource Technol.* 59, 81-96.

Cristiani-Urbina E., Netzahuatl-Mun A. R., Manriquez-Rojas F. J., Jua´rez-Ramı´rez C., Ruiz-Ordaz N., & Galı´ndez-Mayer J. (2000). Batch and fed-batch cultures for the treatment of whey with mixed yeast cultures, *Process Biochemistry* 35, 649–657,

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F., (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 8, 350-366.

Davila-Vazquez G., Felipe Alatriste-Mondrago F., Leo´n-Rodrı´guez A., & Razo-Floresa E. (2008). Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: Influence of initial substrate concentration and pH, *International journal of hydrogen energy* 3 3, 4 9 8 9 – 4 9 9 7

Domingues L. , Teixeira J. A., & Lima N. (1999). Construction of a flocculent Saccharomyces cerevisiae fermenting lactose *Appl Microbiol Biotechnol* , 51: 621-626

Domingues L. , Lima N., & Teixeira J. A.; (2001). Alcohol Production from Cheese Whey Permeate Using Genetically Modified Flocculent Yeast Cells; *Biotechnology and bioengineering*, VOL. 72, NO. 5, 507-514

Duff, S.J.B., & Murray, W.D., (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. *Bioresource Technol*. 55, 1-33.

Echegaray O. F., Carvalho J. C. M., Fernandes A. N. R., Sato S., Aquarone E., & Vitolo M. (2000). Fed-batch culture of Sacchoromyces cerevisiae in sugar-cane blackstrap molasses: invertase activity of intact cells in ethanol fermentation *Biomass and Bioenergy*, V:19, I: 1, 39-50

Fan Z., South C., Lyford K., Munsie J., Walsum P. & Lynd L. R. (2003). Conversion of paper sludge to ethanol in a semicontinuous solids-fed reactor *Bioprocess Biosyst Eng* 26, 93–101

Ferrari, M.D., Loperena, L., & Varela, H. (1994). Ethanol production from concentrated whey permeate using a fed-batch culture of *Kluyveromyces fragilis*. *Biotechnol. Lett*. 16, 205- 210

Geens J., Bruggen B. V., & Vandecasteele C. (2004). Shorter Communication Characterisation ofthe solvent stability ofpolymeric nano$ltration membranes by measurement ofcontact angles and swelling, *Chemical Engineering Science* 59, 1161 – 1164

Gestel T. V., Bruggen B. V., Buekenhoudt A., Dotremont C., Luyten J., Vandecasteele C., & Maesc G. (2003). Surface modification of _-Al2O3/TiO2

multilayer membranes for applications in non-polar organic solvents, *Journal of Membrane Science* 224, 3–10

Ghaly AE, & El-Taweel AA. (1995). Effect of micro-aeration on the growth of *Candida pseudotropicalis* and production of ethanol during batch fermentation of cheese whey. *Bioresource Technol*., 52: 203-217.

Ghaly AE, & El-Taweel AA. (1997). Kinetic modelling of continuous production of ethanol from cheese whey. *Biomass Bioener*.,12: 461-472.

Gong, C.S., Cao, N.J., Du, J., & Tsao, G.T., (1999). Ethanol production from renewable resources. *Advan. Biochem. Eng. / Biotechnol*. 65, 207-241.

Gough S., Flynn O., Hack C. J., & Marchant R. (1996). Fermentation of molasses using a thermotolerant yeast, Kluyveromyces marxianus IMB3: simplex optimisation of media supplements *Appl Microbiol Biotechnol* , 46:187-190

Grba, S., Tomas, V.S., Stanzer, D., Vahcic, N., & Skrlin, A., (2002). Selection of yeast strain *Kluyveromyces marxianus* for alcohol and biomass production on whey. *Chem. Biochem. Eng*. Q. 16, 13-16.

Guadix, A., Sorenson, E., Papageorgiou, L.G., & Guadix, E.M., (2004). Optimal design and operation of ultrafiltration plants. *J Membrane Sci*. 235, 131-138.

Hansen A.C., Zhang Q, Peter W.L. & Lyne P.W.L.; (2005). Review Paper Ethanol–diesel fuel blends—a review *Bioresource Technology* 96 , 277–285

Hari, K.S., Janardhan, R.T., & Chowdary, G.V., (2001). Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. *Bioresource Technol*. 77, 193-196.

Hettenhaus J. R.; (1998). Ethanol fermentation strains present and future requirements for biomass to ethanol commercialization; *United States Department of energy Office of Energy Efficiency and Renewable Energy Ethanol Program and National renewable energy Laboratory*; 1-25

Iwatsubo T., Kusumocahyo S. P., & Shinbo T. (2002). Water/Ethanol Pervaporation Performance of Asymmetric Polyelectrolyte Complex Membrane Constructed by the Diffusion of Poly(acrylic acid) in Chitosan Membrane, *Journal of Applied Polymer Science*, Vol. 86, 265–271

Jurado E., Camacho F., Luzón G., & Vicaria J.M. (2002). A new kinetic model proposed for enzymatic hydrolysis of lactose by a ß-galactosidase from *Kluyveromyces fragilis, Enzyme and Microbial Technology* 31, 300–309

Kargi F., Curme J. A. (1985a). Solid-State Fermentation of sweet sorghum to ethanol in a rotary- drum fermentor, *Biotechnology and Bioeng*. 27, 1122-1125

Kargi F., Curme J. A., & Sheehan J. J. (1985b). Solid-State Fermentation of sweet sorghum, *Biotechnology and Bioeng*. 27, 34-40

Kargi, F., & Ozmihci, S. (2006). Utilization of cheese whey powder for ethanol fermentations: Effects of operating conditions. *Enzyme Microb. Technol*. 38, 711-718.

Kaseno, Miyazawa I., & T. Kokugan (1998). Effect of Product Removal by a Pervaporation on Ethanol Fermentation, *Journal of Fermentation and Bioengineering* 86- 5, 488-493

Kim T.H., Taylor F., & Hicks K.B. (2008). Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment, *Bioresource Technology* 99, 5694–5702

Kourkoutas, Y., Dimitropoulou, S., Kanellaki, M., Marchant, R., Nigam, P., Banat, I.M., & Koutinas, A.A., (2002). High-temperature alcoholic fermentation of whey using *Kluyveromyces marxianus* IMB3 yeast immobilized on delignified cellulosic material. *Bioresource Technol*. 82, 177-181.

Kourkoutas,Y., Psarianos, C., Koutinas, A.A., Kanellaki, M., Banat. I.M., & Marchant, R. (2002). Continuous whey germentation using kefir yeast immobilized on delignified cellulosic material. *J. Agricult. Food Chem*. 50, 2543-2547.

Lark, N., Youkun, X., Qin, C.G., Gong, C.S., & Tsao, G.T., (1997). Production of ethanol from recycled paper sludge using cellulase and yeast,*K.marxianus*.*Biomass Bioener.* 12,135-143.

Lee J.W., Bon-Wook Koo B.W., Choi J.W., Choi D.H., & Choi I.G. (2008). Evaluation of waste mushroom logs as a potential biomass resource for the production of bioethanol *Bioresource Technology* 99, 2736–2741

Lewandowska M., & Kujawski W. (2007). Ethanol production from lactose in a fermentation/pervaporation system *Journal of Food Engineering* 79, 430–437

Lightsey, G.R., (1996). Municipal solid waste processing facility and commercial ethanol production process. *Biotechnology Advances*. 14, 236-257.

Limtong S., Sringiew C., & Yongmanitchai W. (2007). Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated Kluyveromyces marxianus *Bioresource Technology* 98, 3367–3374

Ling K. C., (February 2008). Whey to Ethanol: A Biofuel Role for Dairy Cooperatives? *USDA Rural Business and Cooperative Programs Rural Development Research Report* 214; 1-27

Longhi, L.G.S., Luvizetto, D.J., Ferreira, L.S., Rech, R., Ayub, M.A.Z., & Secchi, A.R., (2004). A growth kinetic model of Kluyveromyces marxianus cultures on cheese whey as a substrate. *J. Indus. Microb. Biotechnol*. 31, 35-40.

Lu X., Li Y., Duan Z., Shi Z., & Mao Z. (2003). A novel, repeated fed –batch, ethanol production system with extremely long term stability achieved by fully recycling fermented supernatants, *Bitechnology Letters* 25: 1819-1826

Lukondeh T, Ashbolt N. J., & Rogers P. L. (2005). Fed-batch fermentation for production of Kluyveromyces marxianus FII 510700 cultivated on a lactose-based medium, *J Ind Microbiol Biotechnol* , , 32: 284–288

Lu X, Li Y, Duan Z, Shi Z, & Mao Z. (2003). A novel, repeated fed-batch, ethanol production system with extremely long term stability achieved by full recycling fermented supernatants. *Biotechnol. Lett*.; 25: 1819-1826.

Mahmoud, M.M., & Kosikowski, F.W., (1982). Alcohol and single-cell protein production by *Kluyveromyces* in concentrated whey permeates with reduced ash. *J Dairy Sci.* 65, 2082-2087.

Maiorella, B.L., & Castillo, F.J., (1984). Ethanol, biomass and enzyme production for whey waste abatement. *Process Biochem*. 8, 157-161.

Mandil C.(Executive Director) IEA; (2004). Biofuels for transport: an International Perspective, *International energy agengy*, April 2004 ; pp 34-40

Marhawa, S.S., & Kennedy, J.F., (1984). Alcohol production from whey permeate by immobilized and free cells of *K. marxianus* NCYC 179. *Process Biochem.* Apr., 79-80.

Marhawa, S.S., Kennedy, J.F., & Shehgal,V.K., (1988). Simulation of process conditions of continuous ethanol fermentation of whey permeate using alginate entrapped *K. marxianus* NCYC 179 cells in apacked-bed reactor system. *Process Biochem*. Feb., 17-22.

Mehaia, M. A., & Cheryan, M., (1984). Holow fibre bioreactor for ethanol production: Application to the conversion of lactose by Kluyveromyces fragilis. *Enzyme Microb.Technol*. 6, 117-120.

Mielenz, J.R., (2001). Ethanol production from biomass: Technology and commercialization status. *Current Opinion in Microbiol*. 4, 324-329.

Moulin, G., Guillaume, M., & Galzy, P., (1980). Alcohol production by yeast in whey ultrafiltrate. *Biotechnol. Bioeng*. 22, 1277-1281.

Najafpour G.D. & Lim J.K. (2002). Evaluation and Isolation of Ethanol Producer Strain SMP-6; 16 th. *Regional Symposium on Chemical Engineering* , 229-236

Natural Gas vehicles for America; (2008). Fact Sheet: Why Ethanol and Biodiesel Alone Can *Not* Achieve 35 Billion Gallons of Petroleum Displacement by 2017;1-8; Retrieved at December 24 2008 from www.ngvc.org/pdfs/35BilGalWhtPaper.pdf

Navajas A., Mallada R., Tellez C., Coronas J., Menhdez M., & Santamaria J. (2002). Preparation of mordenite membranes for pervaporation of water-ethanol mixtures, *Desalination*, 148, 25-29

Nigam, J.N., (2000). Continuous ethanol production from pineapple cannery waste using immobilized yeast cells. *J. Biotechnol*. 80, 189-193.

Ohashı R., Komashita Y., Kishimoto M., & Susukı T. (1998). Continuous production and separation of ethanol without effluence of wastewater using a distiller integrated SCM- reactor system, *Journal of Fermentation and Bioengineering*, V: 86, No:2, 220-225

Oliveira, S. C.; Paiva, T.C.B.; Visconti,. & Giudici R A. E. S. (1999). Continuous alcoholic fermentation process: model considering loss of cell viability. *Bioprocess Eng*.,20, 157-160.

Oliveira, S. C.; DeCastro H. F.; Visconti A. E. S.; & Giudici, R.: (1999). Continuous ethanol fermentation in a tower reactor with flocculating yeast recycle: scale –up effects on process performance, kinetic parameters and model predictions. *Bioprocess Eng*. 20, 525-530.

Ornelas A.P., Silveira W.B., Sampaio F.C. &. Passos F.M.L (2009). The activity of b-galactosidase and lactose metabolism in Kluyveromyces lactis cultured in cheese whey as a function of growth rate *Journal of Applied Microbiology*, 1364-5072

Ozmihci S. & Kargi F. (2007a). Comparison of yeast strains for batch ethanol fermentation of cheese–whey powder (CWP) solution, The Society for Applied Microbiology, *Letters in Applied Microbiology*, 44, 602–606

Ozmihci S. & Kargi F. (2007b). Kinetics of batch ethanol fermentation of cheese-whey powder (CWP) solution as function of substrate and yeast concentrations, *Bioresource Technology*, 98, 2978–2984

Ozmihci S. & Kargi F. (2007c). Ethanol fermentation of cheese whey powder solution by repeated fed-batch operation, *Enzyme and Microbial Technology*, 41, 169–174

Ozmihci S. & Kargi F. (2007d). Continuous ethanol fermentation of cheese whey powder solution: effects of hydraulic residence time, *Bioprocess Biosyst Eng*, 30,79–86

Ozmihci S. & Kargi F. (2007e). Effects of feed sugar concentration on continuous ethanol fermentation of cheese whey powder solution (CWP), *Enzyme and Microbial Technology*, 41, 876–880

Ozmihci S. & Kargi F. (2008). Ethanol production from cheese whey powder solution in a packed column bioreactor at different hydraulic residence times, *Biochemical Engineering Journal*, 42, 180–185

Ozmihci S. & Kargi F. (2009). Fermentation of cheese whey powder solution to ethanol in a packed-column bioreactor: effects of feed sugar concentration, *J Chem Technol Biotechnol*, V 84, N 1, 106-111

Palmqvist, E., Hagerdal, B.H., Galbe, M., Larsson, M., Stenberg, K., Szengyel, Z., Tengborg, C., & Zacchi, G., (1996). Design and operation of a bench scale process development unit for the production of ethanol from lignocellulosics. *Bioresource Technol*. 58, 171-179.

Patle S. , & Lal B. (2008). Investigation of the potential of agro-industrial material as low cost substrate for ethanol production by using Candida tropicalis and Zymomonas mobilis, *Biomass and bioenergy*, 32 , 596 – 602

Renewable Fuel Association (2008). 2007 world fuel ethanol production Retrieved Dember 24 2008 from http://www.ethanolrfa.org/industry/statistics/#E

Reed G. (1981). "*Prescott & Dunn's Industrial Microbiology*" 4th edition

Sa´nchez O.J., & Cardona C.A.; (2008). Review: Trends in biotechnological production of fuel ethanol from different feedstocks *Bioresource Technology* 99, 5270–5295

Shuler, M. L.; & Kargi, F.; (2002). Bioprocess Engineering: Basic Concepts. 2nd edition, Prentice Hall, USA,.

Siso, M.I.G., (1996). The Biotechnological of utilization of cheese whey: a review. *Bioresource Technol*. 57, 1-11.

Silveira WB, Passos FJV, Mantovani HC, & Passos FML. *(*2005). Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: A flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels. *Enzyme Microb. Technol*. 36: 930-936.

Southridge Ethanol, Inc.(2008). *the facts are powerfull* Retrieved December 18, 2008 from http://www.southridgeethanol.com/why_ethanol.php

Spectrum Chemicals & Laboratory Products (2008). Etyl Alcohol (Ethanol) Retrieved December 17, 2008 from http://www.spectrumchemical.com/ethyl-alcohol.aspx)

Tan S. & Ertürk Y. E., (December 2002). T.E.A.E –bakış, A*grarian economic research institute*, I: 1, V: 11, ISSN 1303-8346: 1-4

Tadeusz S.& Carl-Ludwig R., (1990). *Whey and whey utilization* Verlag Th. Mann, Gelsenkirschen- Buer 2nd edition

Telli-Okur M.& Nurdan Eken-Saraçoğlu N., (2008). Fermentation of sunflower seed hull hydrolysate to ethanol by Pichia stipitis, *Bioresource Technology* 99, 2162–2169

Terrel, S.L., Bernard, A., & Bailey, R.B., (1984). Ethanol from whey: continuous fermentation with catabolite repression-resistant *S. cerevisiae* mutant. *Appl. Environ. Microbiol*. 9, 558-580.

Texeira M. R. (2006). Research review paper Endless versatility in the biotechnological applications of Kluyveromyces LAC genes *Biotechnology Advances* 24, 212– 225

Wang, C.J., Jayanata, Y., & Bajpai, R.K.,. (1987). Effect of multiple substrates in ethanol fermentations from cheese whey. *J. Fermen. Technol*. 65, 249-253.

Wilkins M.R., Wilbur W. Widmer W. W., & Grohmann K.; (December 2007). Simultaneous saccharification and fermentation of citrus peel waste by *Saccharomyces cerevisiae* to produce ethanol. *Process Biochemistry* Volume 42, Issue 12, Pages 1614-1619

Yim G. & Glover C. (Sept 2007-Apr-2008). Food microbiology: the bascis and the details of cheese production. *The Science Creative Quarterly*. 2008; Issue 3:1-3

Wikipedia support: a non-profit project. (2008).; Ethanol, December 17, 2008 from http://en.wikipedia.org/wiki/Ethanol

Zafar S., & Owais M., (2006). Ethanol production from crude whey by *Kluyveromyces marxianus. Biochemical Engineering Journal* 27, 295–298

Zayed, G., & Meyer, O., (1996). The single-batch bioconversion of wheat straw to ethanolemploying the fungus Trichoderma viride and the yeast *Pachysolen tannophylus*. *Appl. Microbiol. Biotechnol*. 45, 551-555.

APPENDICES:
RAW EXPERIMENTAL DATA

A.1 Raw Data For Batch Shake Flask Experiments

A. 1.1 Raw Data for Comparison of Different Substrates

Table A 1.1: Comparison of NRRL 1109 with NRRL 1195 in different media: a-CW with *K. marxianus* NRRL-1109, b-CW with *K. marxianus* NRRL-1195, c-CWP with *K. marxianus* NRRL- 1109, d-CWP with *K. marxianus* NRRL-1195, e- Lactose with *K. marxianus* NRRL-1109, f- Lactose with *K. marxianus* NRRL- 1195

(a)

CW with K. marxianus NRRL-1109				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-274	26292	0.00
7	4.77	-190	6486	0.47
24	4.79	-140	406	1.02
31	4.81	-110	340	1.14
48	4.81	-120	75	1.16
55	4.80	-120	75	1.16
72	4.78	-100	48	1.19

(b)

CW with K. marxianus NRRL-1195				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-274	26292	0.00
7	4.79	-185	6558	0.42
24	4.81	-130	511	0.98
31	4.84	-95	410	0.98
48	4.84	-110	112	0.98
55	4.83	-100	110	0.98
72	4.82	-100	56	1.06

Table A 1.1 to be continued

(c)

CWP with K. marxianus NRRL-1109				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.04	-289	26866	0.00
7	4.49	-250	15950	0.44
24	4.50	-163	466	0.90
31	4.48	-100	350	0.90
48	4.48	-120	108	1.20
55	4.49	-120	110	1.75
72	4.50	-120	41	1.78

(d)

CWP with K. marxianus NRRL-1195				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.04	-289	26866	0.00
7	4.54	-218	6665	0.45
24	4.55	-150	493	0.80
31	4.57	-100	290	0.83
48	4.57	-95	114	1.24
55	4.58	-100	108	1.77
72	4.59	-110	59	1.79

(e)

Lactose with K. marxianus NRRL-1109				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-261	28515	0.00
7	3.64	-208	7651	0.21
24	3.60	-147	489	0.25
31	3.54	-95	341	0.40
48	3.54	-110	120	0.45
55	3.54	-110	120	0.65
72	3.52	-120	51	0.81

(f)

Lactose with K. marxianus NRRL-1195				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-261	28515	0.00
7	3.18	-205	7059	0.37
24	3.18	-170	428	0.89
31	3.18	-120	249	0.93
48	3.18	-125	95	0.94
55	3.18	-125	95	0.96
72	3.18	-120	45	1.12

Table A.1.2 Comparison of NRRL-1109 with NRRL-1195 ın Different Media

	CW with NRRL-1109	**CW with NRRL-1195**	**CWP with NRRL-1109**	**CWP with NRRL-1195**	**LAC with NRRL-1109**	**LAC with NRRL- 1195**
$Y_{E/S}$	0.36	0.32	0.52	0.53	0.22	0.31
Final EtOH (%, v v^{-1})	1.19	1.06	1.78	1.79	0.81	1.12
sugar utilization rate (mg l^{-1} h^{-1})	1078.58	1074.19	1100.01	1098.88	1167.77	1170.31
Ethanol formation rate (mg l^{-1} h^{-1})	130.57	116.30	195.30	196.40	88.87	122.89

Table A.1.3 Raw Data on Ethanol Fermentation Performance of Different Kluyveromyces Marxianus Strains from CWP Solution

***K. marxianus* NRRL-1195**					***K. marxianus* DSMZ-7239**					**Kontrol**				
Hour	pH	ORP	Total sugar (mg l^{-1})	Ethanol (ml/100ml)	Hour	pH	ORP	Total sugar (mg l^{-1})	Ethanol (ml/100ml)	Hour	pH	ORP	Total sugar (mg l^{-1})	Ethanol (ml/100ml)
0	5.00	-90	49940	0.00	0	5.00	-90	49940	0.00	0	5.00	-75	49940	0.00
17	4.90	-280	46500	0.86	17	4.81	-290	37503	1.98	89	4.88	-90	46395	0.00
24	4.65	-290	46330	1.18	24	4.54	-320	28738	2.86					
41	4.13	-200	35546	2.77	41	4.44	-250	9754	3.60					
48	4.06	-180	32300	3.00	48	4.48	-250	7500	3.56					
65	4.10	-160	14456	3.02	65	4.54	-250	6456	3.53					
72	4.15	-155	12056	3.03	72	4.60	-250	3848	3.47					
89	4.15	-155	2117	3.10	89	4.60	-250	1095	3.35					

Table A 1.4 Raw Data for Product Yield Coefficient, Final Ethanol, Specific Sugar Utilization and Ethanol Formation Rate for Different *Kluyveromyces Marxianus* Strains Fermenting CWP Solution

	$Y_{E/S}$	**Final EtOH**	**Specific EtOH form. Rate (ml $g^{-1}h^{-1}$)**	**Specific sugar utilization rate (mg $g^{-1}h^{-1}$)**	**Sugar utilization rate (mg $l^{-1}h^{-1}$)**	**Ethanol formation rate (ml $l^{-1}h^{-1}$)**
***K. marxianus* NRRL-1195**	0.5	3.1	2.89	2487.67	537.34	0.63
***K. marxianus* DSMZ-7239**	0.54	3.35	4.07	2540.83	548.82	0.88

Table A 1.5. Raw Data for The Effects of Initial pH on Ethanol Fermentation of CWP Solution

pH=3				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	2.99	-275	34653	0.00
7	2.98	-248	22590	0.44
24	2.93	-257	17520	0.95
31	2.94	-244	3948	1.02
48	2.92	-235	606	1.06
55	2.93	-215	580	1.06
72	2.92	-200	98	1.06

pH=4				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	4.07	-265	34066	0.00
7	4.00	-245	25405	0.45
24	3.87	-250	18693	1.03
31	3.87	-238	7969	1.13
48	3.80	-205	204	1.15
55	3.92	-195	140	1.15
72	3.90	-180	76	1.18

pH=5				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.07	-285	34443	0.00
7	4.50	-205	24208	0.50
24	4.30	-210	15425	0.85
31	4.35	-222	8221	1.26
48	4.36	-200	648	1.26
55	4.35	-190	450	1.26
72	4.44	-180	53	1.28

pH=6				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.03	-290	28621	0.00
7	4.85	-180	16506	0.48
24	4.55	-200	12334	1.16
31	4.56	-186	7718	1.20
48	4.60	-190	394	1.25
55	4.61	-195	350	1.25
72	4.65	-180	165	1.26

Table A 1.5 to be continued

pH =7

Hour	pH	ORP	Total sugar (mg l^{-1})	Ethanol (ml/100ml)
0	7.11	-300	31134	0.00
7	5.12	-200	25952	0.40
24	4.88	-200	10952	1.01
31	4.82	-155	6629	1.14
48	4.85	-150	405	1.14
55	4.86	-150	400	1.14
72	5.05	-145	101	1.14

Table A 1.6. Raw data of product yield coefficient, final ethanol, sugar utilization and ethanol formation rate at different initial pHs

pH	**7**	**6**	**5**	**4**	**3**
$Y_{E/S}$	0.29	0.35	0.29	0.27	0.24
Sugar utilization rate (mg $l^{-1}h^{-1}$) t=48 hours	640.19	588.06	704.06	705.46	709.31
final EtOH	1.14	1.26	1.28	1.18	1.06
EtOH form. Rate (ml l^{-1} h^{-1})	0.16	0.18	0.18	0.16	0.15

Table A1.7 Raw Data for Ethanol Fermentation of CWP Solution at Different Initial ORP s

Na-thioglycolate 50 mg l^{-1}					*Na-thioglycolate 100 mg l^{-1}*				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Hour**	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-20	46886	0.00	0	5.00	-80	46886	0.00
7	4.88	-58	41867	0.72	7	4.85	-103	45976	0.55
24	4.85	-55	15364	0.98	24	4.85	-100	12409	2.20
31	4.85	-62	14300	1.50	31	4.85	-100	12300	2.25
48	4.87	-65	14250	1.80	48	4.84	-120	12300	2.48
55	4.87	-80	13800	1.90	55	4.85	-164	12150	2.49
72	4.97	-85	13064	2.10	72	4.94	-170	12009	2.56
137	6.60	-80		2.20	137	5.50	-197		2.26

Na-thioglycolate 200 mg l^{-1}					*Na-thioglycolate 250 mg l^{-1}*				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Hour**	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	5.00	-140	46886	0.00	0	5.00	-156	46886	0.00
7	4.85	-121	43431	1.39	7	4.84	-130	44340	1.45
24	4.84	-119	10372	3.17	24	4.83	-155	13136	2.10
31	4.85	-123	7245	3.20	31	4.84	-187	12820	2.30
48	4.85	-150	6850	3.28	48	4.85	-205	12540	2.58
55	4.86	-250	6049	3.50	55	4.85	-273	12031	3.25
72	4.95	-250	5280	3.63	72	4.95	-280	11609	3.20
137	4.90	-216	120	3.65	137	5.03	-280.7	100	3.40

Table A1.7 to be continued

Na-thioglycolate 300 mg l^{-1}

Hour	pH	ORP	Total sugar (mg l^{-1})	Ethanol (ml/100ml)
0	5.00	-163	46886	0.00
7	4.83	-236	41503	2.38
24	4.83	-263	9281	2.64
31	4.84	-237	4372	2.60
48	4.85	-258	4300	2.62
55	4.85	-294	4298	2.83
72	4.95	-295	4117	3.20
137	5.10	-297.7	90	3.61

Table A 1.8 Raw Data for the Effects of External Nutrients Additions on CWP Fermentation

CWP			***CWP, 2N,P***		***CWP, 4N,P***		***CWP, N, 2P***	
Hour	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	26500	0.00	22232	0.00	26252	0.00	23737	0.00
7	24250	0.47	21800	0.00	24500	0.00	21285	0.00
24	19707	0.84	21337	0.00	19707	0.00	18264	0.00
31	6903	1.16	20048	0.00	15248	0.01	14250	0.01
48	2048	1.24	10248	0.01	10550	0.01	11248	0.01
55	1250	1.25	9208	0.09	9721	0.01	9105	0.02
72	1138	1.26	8160	0.42	8905	0.02	8202	0.49
96	1000	1.27	8150	0.50	8500	0.34	8000	0.73

CWP, N, 4P			***CWP, 2N, 2P***		***CWP, 4N, 4P***	
Hour	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	24096	0.00	25476	0.00	24862	0.00
7	23684	0.00	23285	0.00	21648	0.00
24	16697	0.00	16258	0.00	20466	0.00
31	13990	0.01	15150	0.01	18200	0.01
48	9970	0.01	13480	0.01	15678	0.01
55	8200	0.32	12500	0.05	12198	0.01
72	7693	0.50	7328	0.32	7328	0.26
96	7550	0.50	6805	0.40	7058	0.31

Table A 1.9 Raw Data for Product Yield Coefficient and Final Ethanol for Effects of External Nutrients Additions

	$Y_{E/S}$	EtOH final	EtOH formation rate (ml $l^{-1}h^{-1}$)	Sugar utilization rate (mg $l^{-1}h^{-1}$)
CWP	0.39	1	0.13	266
CWP, 2N,P	0.28	1	0.05	147
CWP, 4N,P	0.15	0	0.04	185
CWP, N, 2P	0.37	1	0.08	164
CWP, N, 4P	0.24	1	0.05	172
CWP, 2N, 2P	0.17	0	0.04	194
CWP, 4N, 4P	0.14	0	0.03	185

Table A 1.10 Raw Data for the Effects of CWP Concentration on Ethanol Fermentation Using *K. Marxianus* NRRL-1195

CWP 52 g l^{-1}				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.53	-85	25632	0.00
7	6.05	-138	21510	0.00
24	4.27	-185	20590	0.28
31	4.20	-130	20297	0.29
48	4.10	-175	8332	0.94
55	4.10	-175	8000	0.94
72	4.00	-175	4259	1.11
144	3.84	-175	453	1.55
168	3.84	-175	450	1.55
216	3.84	-175	450	1.55

CWP 104 g l^{-1}				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.41	-252	47966	0.000
7	6.10	-99	46415	0.000
24	4.65	-155	44378	0.063
31	4.60	-150	44249	0.620
48	4.50	-155	24556	0.780
55	4.20	-150	23500	0.800
72	4.10	-125	2901	2.190
144	4.10	-120	731	2.190
168	4.10	-120	730	2.19
216	4.10	-120	730	2.19

CWP 156 g l^{-1}				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.32	-175	74496	0.00
7	6.12	-122	73533	0.00
24	4.91	-185	66949	0.00
31	4.70	-180	66092	0.20
48	4.30	-170	66000	0.20
55	4.20	-170	60249	0.25

CWP 208 g l^{-1}				
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.25	-175	103596	0.00
7	6.09	-122	89147	0.00
24	5.11	-185	87497	0.02
31	5.10	-180	86899	1.01
48	4.50	-170	85262	1.03
55	4.40	-170	80124	1.10

Table A 1.10 to be continued

72	4.10	-175	5630	2.44
144	4.01	-170	915	3.06
168	4.00	-165	900	3.10
216	4.00	-170	850	3.10

CWP 260 g l^{-1}

Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.07	-150	122949	0.00
7	6.07	-121	120645	0.00
24	5.29	-180	117130	0.00
31	5.10	-170	116851	0.02
48	4.45	-150	116124	0.63
55	4.35	-100	96851	0.75
72	4.15	-150	85484	1.62
144	4.15	-155	7274	3.72
168	4.10	-150	7259	4.64
216	4.00	-145	2500	7.11

72	4.25	-175	50203	6.10
144	4.14	-170	3456	6.20
168	4.10	-165	3819	6.24
216	4.00	-150	3500	6.24

CWP 312 g l^{-1}

Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Ethanol (ml/100ml)**
0	6.10	-168	145939	0.00
7	6.07	-118	144594	0.00
24	5.33	-197	132409	0.00
31	5.00	-180	130678	0.38
48	4.55	-170	129985	1.44
55	4.30	-170	129900	1.40
72	4.15	-170	129184	1.36
144	4.10	-160	8644	4.50
168	4.00	-150	8656	8.22
216	4.00	-145	2590	10.59

Table A 1.11 Raw Data for Product Yield Coefficient, Percent Sugar Utilization, Sugar Utilization Rate and Overall Ethanol Formation Rate with Variable CWP Concentration Using *K. Marxianus* NRRL-1195

CWP concentration ($g\ l^{-1}$)	**52**	**104**	**156**	**208**	**260**	**312**
$Y_{E/S}$	0.49	0.37	0.33	0.49	0.47	0.54
Y_T	0.54	0.54	0.54	0.54	0.54	0.54
Y_E/Y_T	0.90	0.68	0.62	0.91	0.86	1.00
Percent $EtOH_{final}$	1.55	2.190	3.10	6.24	7.11	10.59
% final sugar utilization	98.23	98.47	98.85	96.62	97.96	98.22
Sugar utilization rate ($mgl^{-1}h^{-1}$)	116.57	218.68	340.95	463.41	557.63	663.65
overall EtOH formation rate ($ml\ l^{-1}h^{-1}$)	0.07	0.1	0.14	0.29	0.33	0.49

Table A 1.12 Raw Data for the Effects of CWP Concentration on Ethanol Fermentation Using *K. Marxianus* DSMZ-7239

CWP (52 g l^{-1})						***CWP (104 g l^{-1})***					
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**	**Hour**	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**
0	5.06	-150	25890	0	0	0	5.13	-160	49940	0.00	0.000
7	4.80	-180	21850	15.60	0.59	7	5.10	-200	37503	24.90	0.640
24	4.85	-190	21440	17.19	0.90	24	4.80	-250	28738	42.45	0.720
31	4.86	-238	8210	68.29	1.17	31	4.64	-327	9754	80.47	1.100
48	4.67	-240	3240	87.49	1.94	48	4.53	-330	3500	92.99	3.590
55	4.60	-250	210	99.19	2.40	55	4.50	-320	2458	95.08	3.680
72	4.54	-250	150	99.42	1.74	72	4.47	-340	1456	97.08	3.420
168	4.50	-308	130	99.50	1.71	168	5.90	-318	465	99.07	3.380
CWP (156 g l^{-1})						***CWP (208 g l^{-1})***					
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**	**Hour**	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**
0	5.19	-145	75896	0	0.00	0	5.08	-155	104568	0	0.00
7	5.15	-200	72564	4.39	0.55	7	4.98	-230	90546	13.41	0.57
24	4.95	-250	70213	7.49	1.25	24	4.95	-280	84257	19.42	1.28
31	4.78	-342	65894	13.18	1.43	31	4.86	-355	80451	23.06	1.56
48	4.73	-350	60789	19.90	3.10	48	4.82	-370	74568	28.69	2.95
55	4.65	-340	45265	40.36	3.56	55	4.78	-350	74520	28.74	2.98
72	4.55	-320	3456	95.45	5.10	72	4.70	-340	47584	54.49	3.62
168	5.90	-330	785	98.97	5.10	168	4.80	-348	5978	94.28	5.60

Table A 1.12 to be continued

CWP (260 g l^{-1})						*CWP (312 g l^{-1})*					
Hour	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**	**Hour**	**pH**	**ORP**	**Total sugar (mg l^{-1})**	**Sugar conversion**	**Ethanol (ml/100ml)**
0	5.06	-160	126751	0	0.00	0	5.16	-165	145250	0	0.00
7	5.01	-240	120423	4.99	0.49	7	5.05	-225	144785	0.32	0.45
24	5.01	-290	100452	20.75	1.29	24	5.06	-303	130546	10.12	1.15
31	4.92	-351	85475	32.56	1.57	31	5.07	-373	130125	10.41	1.20
48	4.86	-375	78412	38.14	1.90	48	5.03	-375	115142	20.73	1.27
55	4.80	-360	78632	37.96	2.02	55	5.00	-360	110258	24.09	1.33
72	4.73	-340	77456	38.89	3.10	72	4.95	-340	100045	31.12	1.51
168	4.60	-353	9000	92.90	4.10	168	4.79	-354	10475	92.79	4.84
192	4.60	-350	5421	95.72	4.10	192	4.79	-350	10245	92.95	4.85

Table A 1.13 Raw Data for Product Yield Coefficient, Percent Ethanol Production, Sugar Utilization Rate and Overall Ethanol Formation Rate at Different CWP Concentrations with *K. Marxianus* DSMZ-7239 Experiments

CWP concentration (g l^{-1})	**52**	**104**	**156**	**208**	**260**	**312**
$Y_{E/S}$	0.53	0.54	0.54	0.45	0.28	0.28
Percent $EtOH_{final}$	1.74	3.42	5.10	3.62	3.10	1.51
Sugar utilization rate (mg $l^{-1}h^{-1}$)	357.50	673.39	1006.11	791.44	684.65	627.85
Specific sugar utilization rate (mg g^{-1} h^{-1})	715.00	1346.78	2012.22	1582.89	1369.31	1255.69
EtOH formation rate (ml l^{-1} h^{-1})	0.24	0.47	0.71	0.50	0.43	0.21
Specific EtOH formation rate(ml g^{-1} h^{-1})	0.48	0.94	1.42	1.01	0.73	0.42

Table A 1.14 Raw Data of Effects of Initial Biomass (Yeast) Concentration on Ethanol Yields

X (mg l^{-1})	**170**	**340**	**510**	**680**	**850**	**1020**
$Y_{E/S}$	0.52	0.53	0.53	0.53	0.54	0.54
Percent EtOH	3.10	3.27	3.30	3.41	3.60	3.63
EtOH formation rate (ml $l^{-1}h^{-1}$) 120 h	0.2583	0.2725	0.2750	0.2842	0.3000	0.3025
Sugar utilization rate (mg $l^{-1}h^{-1}$)	952.85	991.88	1566.55	1608.42	2144.38	2199.88

A.2 Raw Data for the Repeated Fed-Batch Experiments

A. 2.1 Raw Data for Different Feed CWP Concentrations

Table A 2.1 Raw Data of Fed-Batch Experiments with the Feed Sugar 50 g l^{-1}

Time (h)	pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (%)	Biomass (g l^{-1})
				Control	Experiment		
run 1 0	4.48	-68	1000		979	0.75	5.1
1	4.51	-211	1074	5661	1782	0.75	
2	4.48	-210	1148	9669	949	0.75	
3	4.42	-180	1222	13140	1027	0.75	
4	4.36	-150	1296	16174	827	0.85	
5	4.38	-170	1370	18849	4917	1.06	
6	4.72	-180	1444	21225	14191	1.1	
7	4.76	-190	1518	23350	23010	1.1	
8	4.76	-190	1592	25262	25211	1.11	
24	4.72	-117	2926	41365	31720	1.12	5.82
25	4.72	-120	3000	41907	35248	1.2	
26	4.71	-125	3074	42421	35157	1.25	
27	4.71	-130	3148	42909	35250	1.28	
28	4.71	-140	3222	43373	38450	1.3	
29	4.71	-150	3296	43813	39000	1.31	
30	4.71	-160	3370	44233	39150	1.33	
48	4.71	-165	3444	49390	39100	1.35	6.54
batch	4.71	-160	-		905	1.4	
run 2 0	4.56	-150	1676		850	1.37	6.54
1	4.57	-150	1750	3597	1505	1.39	
2	4.57	-160	1824	6094	1510	1.4	
3	4.58	-170	1898	8373	1720	1.42	
4	4.59	-170	1972	10462	1750	1.45	
5	4.6	-180	2046	12383	3800	1.48	
6	4.6	-185	2120	14156	5685	1.5	
7	4.6	-190	2194	15798	6800	1.52	
8	4.6	-195	2268	17322	7425	1.5	
24	4.71	-198	3602	32278	30400	1.52	7.6944
25	4.71	-199	3676	32859	31248	1.53	
26	4.7	-200	3750	33415	33157	1.54	
27	4.71	-201	3824	33947	33010	1.55	
28	4.71	-210	3898	34457	34450	1.55	

Table A 2.1 to be continued

	29	4.71	-220	3972	34946	34650	1.55	
	30	4.71	-210	4046	35416	35400	1.55	
	48	4.61	-278	5548	41506	39805	1.55	8.85
batch		4.51	-270	-		859	1.56	
run 3	0	4.5	-290	2000		865	1.37	8.85
	1	4.51	-295	2074	3218	4844	1.37	
	2	4.51	-298	2148	5389	1008	1.37	
	3	4.51	-300	2222	7398	1060	1.37	
	4	4.51	-302	2296	9263	1095	1.38	
	5	4.52	-301	2370	10998	1105	1.38	
	6	4.52	-301	2444	12617	1200	1.38	
	7	4.52	-301	2518	14130	1340	1.38	
	8	4.53	-308	2592	15549	1528	1.38	
	24	4.55	-290	3926	30174	4910	1.81	9.0492
	25	4.55	-295	4000	30770	5117	1.85	
	26	4.55	-290	4074	31341	4329	1.9	
	27	4.55	-285	4148	31891	4777	1.91	
	28	4.56	-280	4222	32419	4941	1.95	
	29	4.56	-285	4296	32927	5102	1.98	
	30	4.56	-291	4370	33416	5208	1.99	
	48	4.57	-292	5872	39892	8699	2.05	9.25
batch		4.36	-276			568	2.1	
run 4	0	4.29	-236	2000		560	2	9.25
	1	4.3	-230	2074	2868	1051	2.05	
	2	4.31	-231	2148	4998	1008	2.15	
	3	4.31	-235	2222	6968	1089	2.18	
	4	4.31	-230	2296	8797	1099	2.22	
	5	4.29	-230	2370	10499	1182	2.28	
	6	4.25	-215	2444	12087	1243	2.34	
	7	4.25	-210	2518	13571	1341	2.37	
	8	4.24	-215	2592	14962	1568	2.5	
	24	4.16	-215	3926	29308	3782	3.4	9.25
	25	4.17	-276	4000	29892	3795	3.49	
	26	4.17	-300	4074	30453	3587	3.5	
	27	4.17	-305	4148	30991	3420	3.54	
	28	4.17	-310	4222	31509	3100	3.55	
	29	4.17	-315	4296	32008	3964	3.57	
	30	4.17	-305	4370	32488	3220	3.58	
	48	4.17	-300	5872	38839	2699	3.8	9.23
batch		4.18	-280			1100	3.88	

Table A 2.1 to be continued

run 5 0	4.29	-236	2000		1136	2.25	9.23
1	4.3	-230	2074	3353	1885	2.26	
2	4.31	-231	2148	5399	1441	2.28	
3	4.31	-235	2222	7292	1305	2.3	
4	4.31	-230	2296	9049	1350	2.34	
5	4.29	-230	2370	10684	1295	2.35	
6	4.25	-215	2444	12209	1305	2.37	
7	4.25	-210	2518	13635	1310	2.4	
8	4.24	-215	2592	14972	1340	2.5	
24	4.16	-215	3926	28753	1161	3.12	9.23
25	4.17	-276	4000	29314	1108	3.23	
26	4.17	-300	4074	29853	1154	3.25	
27	4.17	-305	4148	30370	1185	3.24	
28	4.17	-310	4222	30868	1280	3.25	
29	4.17	-315	4296	31347	1292	3.27	
30	4.17	-305	4370	31808	1295	3.27	
48	4.17	-300	5872	37909	1927	3.72	9.23
batch	4.18	-280			148	3.8	

Table A 2.2 RAW Data of Fed-Batch Experiments with the CWP Containing 75 g l^{-1} Total Sugar

Time (h)	pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (v v^{-1})	Biomass (g l^{-1})
				Control	Experiment		
run 1 0	4.2	-155	1000		7541	0	8.44
1	4.29	-150	1074	12890	8600	0	
2	4.3	-160	1148	17501	10542	0	
3	4.31	-175	1222	21517	16580	0	
4	4.35	-180	1296	25046	18649	0	
5	4.36	-195	1370	28172	20489	0.30	
6	4.37	-200	1444	30960	28623	0.30	
7	4.44	-205	1518	33462	30500	0.30	
8	4.44	-205	1592	35720	30500		
24	4.5	-255	2926	55021	50454	0.32	
25	4.52	-268	3000	55680	50990	0.32	
26	4.52	-216	3074	56306	51000	0.34	
27	4.52	-216	3148	56899	52789	0.35	
28	4.5	-200	3222	57463	52450	0.35	
29	4.48	-215	3296	58000	53456	0.35	
30	4.44	-218	3370	58512	53873	0.35	
48	4.44	-218	3444	64831	55250	0.35	
batch	4.46	-361	-		15108	0.35	
run 2 0	4.25	-332	2000		13678	0	8.5
1	4.25	-300	2074	16165	15440	0	
2	4.25	-280	2148	18467	16560	0	
3	4.25	-275	2222	20605	16780	0	
4	4.2	-280	2296	22595	18450	0	
5	4.21	-259	2370	24453	20560	0	
6	4.2	-250	2444	26191	23548	0	
7	4.22	-245	2518	27820	24670	0	
8	4.22	-245	2592	29350	24670		
24	4.22	-230	3926	45343	35790	0.6	
25	4.2	-220	4000	46003	33670	0.65	
26	4.2	-225	4074	46636	35230	0.68	
27	4.2	-230	4148	47246	36890	0.75	
28	4.2	-240	4222	47832	37564	0.78	
29	4.22	-240	4296	48397	36990	0.8	
30	4.22	-235	4370	48941	36450	0.8	
48	4.08	-230	5872	56187	32758	0.8	

Table A 2.2 to be continued

	batch	4.05	-235	-		13450	1.63	
run 3	0	4.05	-244	2000		10340	1.45	8.8
	1	4.15	-234	2074	12860	10340	1.45	
	2	4.2	-232	2148	15194	11234	1.45	
	3	4.22	-230	2222	17361	12670	1.5	
	4	4.24	-200	2296	19378	13890	1.55	
	5	4.26	-205	2370	21261	16745	1.56	
	6	4.28	-185	2444	23022	18590	1.58	
	7	4.3	-185	2518	24674	18690	1.6	
	8	4.3	-185	2592	26225	18690	1.6	
	24	4.33	-175	3926	42434	16780	3.12	
	25	4.33	-170	4000	43103	15870	3.2	
	26	4.35	-170	4074	43745	16890	3.3	
	27	4.34	-170	4148	44363	16550	3.3	
	28	4.35	-165	4222	44957	16500	3.4	
	29	4.36	-160	4296	45529	16000	3.4	
	30	4.4	-160	4370	46081	15400	3.4	
	48	4.4	-155	5872	53425	15000	3.8	
	batch	4.2	-155			9540	4	
run 4	0	4.15	-290	2000		9200	3.45	8.9
	1	4.15	-256	2074	11884	9340	3.45	
	2	4.2	-245	2148	14370	12000	3.45	
	3	4.25	-233	2222	16678	12500	3.45	
	4	4.24	-200	2296	18826	12890	3.45	
	5	4.26	-205	2370	20832	12900	3.45	
	6	4.2	-185	2444	22708	15780	3.45	
	7	4.2	-165	2518	24467	15990	3.45	
	8	4.3	-165	2592	26119	15990	3.45	
	24	4.3	-155	3926	43383	21000	3.55	
	25	4.3	-170	4000	44095	21890	3.6	
	26	4.35	-170	4074	44779	21560	3.64	
	27	4.3	-170	4148	45437	21450	3.65	
	28	4.3	-145	4222	46070	21567	3.79	
	29	4.4	-140	4296	46680	21880	3.8	
	30	4.4	-140	4370	47267	21345	3.85	
	48	4.4	-155	5872	55089	21780	3.88	
	batch	4.4	-255			10453	3.9	

Table A 2.2 to be continued

run 5	0	4.3	-223	2000		8500	3	9.1
	1	4.35	-231	2074	10968	8800	3.1	
	2	4.3	-212	2148	13253	8850	3.1	
	3	4.22	-200	2222	15375	8900	3.1	
	4	4.2	-200	2296	17351	9000	3.1	
	5	4.2	-195	2370	19195	9500	3.1	
	6	4.2	-195	2444	20920	9456	3.2	
	7	4.3	-185	2518	22537	9560	3.2	
	8	4.3	-185	2592	24056	9560	3.2	
	24	4.3	-175	3926	39930	15460	3.6	
	25	4.35	-170	4000	40585	16780	3.65	
	26	4.35	-170	4074	41214	16000	3.66	
	27	4.34	-170	4148	41819	16050	3.68	
	28	4.35	-145	4222	42401	15680	3.7	
	29	4.3	-180	4296	42962	15460	3.8	
	30	4.3	-190	4370	43502	15450	3.8	
	48	4.3	-195	5872	50694	15600	4.5	
	batch	4.1	-278			10300	3.8	

Table A 2.3 Raw Data of Fed-Batch Experiments with the CWP Containing 100 g l^{-1} Total Sugar

Time (h)		pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (%)	Biomass (g l^{-1})
					Control	Experiment		
run 1	0	3.6	-130	1000		18710	0.9	8.64
	1	4.15	-153	1074	25468	22520	0.9	
	2	4.18	-155	1148	31254	28456	1.09	
	3	4.26	-165	1222	36263	32545	1.2	
	4	4.35	-208	1296	40643	35420	1.55	
	5	4.44	-190	1370	44504	28450	1.92	
	6	4.53	-189	1444	47934	25120	2.4	
	7	4.53	-170	1518	51001	25000	2.6	
	8	4.55	-180	1592	53761	24100	2.79	
	24	4.7	-260	2926	77004	45850	3.11	5.24
	25	4.7	-250	3000	77787	45000	3.15	
	26	4.7	-240	3074	78529	46213	3.15	
	27	4.7	-243	3148	79233	47120	3.18	
	28	4.71	-233	3222	79902	46540	3.2	
	29	4.71	-205	3296	80538	45560	3.2	
	30	4.72	-200	3370	81144	45500	3.24	
	48	4.72	-205	3444	88588	45120	3.24	5.25
	batch	4.34	-312	-		15740	3.3	
run 2	0	4.34	-153	2000		15000	1.65	5.9
	1	4.34	-163	2074	18583	16500	1.65	
	2	4.34	-160	2148	21888	20540	1.13	
	3	4.34	-175	2222	24946	20645	1.45	
	4	4.35	-175	2296	27784	24000	0.89	
	5	4.38	-170	2370	30426	24560	0.95	
	6	4.4	-180	2444	32890	25450	0.9	
	7	4.42	-180	2518	35194	26980	0.85	
	8	4.43	-189	2592	37354	27420	0.85	
	24	4.52	-185	3926	59618	34520	0.85	5.96
	25	4.53	-170	4000	60525	35460	0.85	
	26	4.55	-186	4074	61395	38456	0.8	
	27	4.55	-185	4148	62232	39452	0.85	
	28	4.55	-190	4222	63035	37560	0.9	
	29	4.56	-195	4296	63809	38450	0.92	
	30	4.58	-190	4370	64554	39450	0.95	
	48	4.62	-200	5872	74412	40890	0.99	6.46
	batch	4.54	-315	-		20410	1.08	
run 3	0	4.54	-219	2000		18542	0.76	6.34

Table A 2.3 to be continued

1	4.54	-200	2074	21928	20450	0.89	
2	4.54	-210	2148	25052	20560	0.9	
3	4.55	-205	2222	27942	21789	0.95	
4	4.55	-205	2296	30625	22478	0.95	
5	4.55	-205	2370	33121	24560	0.99	
6	4.55	-200	2444	35450	24890	1.08	
7	4.56	-190	2518	37628	26140	1.08	
8	4.56	-195	2592	39669	26780	1.2	
24	4.59	-210	3926	60711	18540	3.33	8.3
25	4.6	-200	4000	61568	18450	3.35	
26	4.62	-190	4074	62391	18500	3.4	
27	4.64	-208	4148	63181	19450	3.45	
28	4.64	-210	4222	63941	23650	3.5	
29	4.64	-215	4296	64672	24503	3.65	
30	4.64	-215	4370	65376	25000	3.65	
48	4.64	-240	5872	74693	25450	3.68	8.21
batch	4.64	-320			14500	3.81	
run 4 0	4.52	-180	2000		13850		5.86
1	4.52	-185	2074	17842	16450	2.49	
2	4.53	-190	2148	21524	17450	2.5	
3	4.53	-180	2222	24931	18450	2.52	
4	4.53	-180	2296	28094	18900	2.55	
5	4.53	-190	2370	31037	20450	2.65	
6	4.55	-185	2444	33782	21450	2.65	
7	4.55	-185	2518	36350	22653	2.79	
8	4.56	-180	2592	38755	24780	3.14	
24	4.61	-190	3926	63562	30560	3.83	6.04
25	4.61	-190	4000	64572	32545	3.93	
26	4.61	-190	4074	65542	32850	4.01	
27	4.66	-180	4148	66474	33450	4.45	
28	4.66	-150	4222	67369	34500	4.5	
29	4.65	-140	4296	68231	35600	4.58	
30	4.66	-140	4370	69061	35890	4.9	
48	4.66	-140	5872	80044	10780	5.16	6.54
batch	4.48	-320			10410	5.22	
run 5 0	4.47	-325	2000		10200	4.55	6.34
1	4.48	-300	2074	14433	14000	4.55	
2	4.48	-295	2148	18339	16500	4.56	
3	4.49	-280	2222	21953	16420	4.56	
4	4.496	-240	2296	25307	16200	4.56	

Table A 2.3 to be continued

5	4.5	-235	2370	28428	16000	4.6	
6	4.5	-230	2444	31340	16420	4.62	
7	4.5	-235	2518	34063	16480	4.63	
8	4.54	-230	2592	36615	16900	4.65	
24	4.61	-238	3926	62924	20560	5.6	8.26
25	4.62	-235	4000	63996	22450	5.62	
26	4.62	-210	4074	65024	23450	5.61	
27	4.62	-200	4148	66013	25123	5.62	
28	4.63	-210	4222	66963	26780	5.61	
29	4.64	-250	4296	67877	26500	5.6	
30	4.65	-240	4370	68757	26450	5.62	
48	4.65	-235	5872	80406	26480	6.8	8.26
batch	4.4	-310			12780	6.8	

Table A 2.4 Raw Data of Fed-Batch Experiments with CWP Containing 125 g l^{-1} Total Sugar

Time (h)		pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (v v^{-1})	Biomass (g l^{-1})
					Control	Experiment		
run 1	0	4.3	-314	1000		33941	2.10	4.94
	1	4.3	-300	1074	40429	38567	2.23	
	2	4.32	-289	1148	45985	40890	2.56	
	3	4.33	-280	1222	50794	42678	2.60	
	4	4.36	-275	1296	54999	42211	2.61	
	5	4.38	-270	1370	58707	43944	2.66	
	6	4.38	-250	1444	62000	41230	2.68	
	7	4.36	-245	1518	64945	38786	2.70	
	8	4.35	-211	1592	67594	35180	2.70	
	24	4.38	-200	2876	89910	40958	5.90	5.36
	25	4.38	-210	2950	90662	41230	5.91	
	26	4.37	-210	3024	91375	44550	5.95	
	27	4.38	-215	3098	92051	45000	5.96	
	28	4.35	-216	3172	92693	46320	5.95	
	29	4.38	-215	3246	93303	45673	5.95	
	30	4.38	-230	3320	93885	44530	5.95	
	48	4.35	-235	4772	101032	30230	5.95	5.34
	batch	4.3	-290	-		25340	5.95	
run 2	0	4.23	-300	2000		25340	5.50	5.4
	1	4.24	-225	2074	29758	29500	5.45	
	2	4.24	-220	2148	33833	30456	5.40	
	3	4.25	-200	2222	37605	36540	5.40	
	4	4.28	-210	2296	41105	40123	5.40	
	5	4.32	-220	2370	44362	42341	5.40	
	6	4.34	-215	2444	47400	43000	5.40	
	7	4.36	-200	2518	50242	48769	5.40	
	8	4.36	-205	2592	52905	50754	5.40	
	24	4.42	-225	3876	80359	75345	5.40	5.35
	25	4.42	-225	3950	81478	77460	5.40	
	26	4.43	-225	4024	82551	77890	5.40	
	27	4.45	-200	4098	83582	78564	5.40	
	28	4.45	-220	4172	84573	78990	5.40	
	29	4.45	-225	4246	85527	82345	5.40	
	30	4.45	-225	4320	86446	82999	5.40	
	48	4.43	-225	5772	98602	90080	5.40	5.32

Table A 2.4 to be continued

	batch	4.42	-342	-		88759	5.40	
	batch	4.44	-322			25673		
run 3	0	4.2	-354	2000		25673	1.37	7.59
	1	4.22	-285	2074	29381	26435	1.50	
	2	4.23	-280	2148	32802	28780	1.77	
	3	4.27	-280	2222	35968	32453	1.98	
	4	4.28	-270	2296	38906	35467	2.10	
	5	4.28	-255	2370	41640	38760	2.11	
	6	4.28	-245	2444	44190	39780	2.11	
	7	4.28	-220	2518	46575	40786	2.15	
	8	4.28	-220	2592	48810	43222	2.15	
	24	4.38	-210	3876	71856	60452	2.77	7.61
	25	4.38	-200	3950	72794	62132	2.79	
	26	4.38	-200	4024	73695	62340	2.80	
	27	4.38	-210	4098	74561	62786	2.80	
	28	4.38	-211	4172	75393	62134	2.80	
	29	4.4	-211	4246	76194	62786	2.80	
	30	4.41	-215	4320	76964	62990	2.88	
	48	4.44	-215	5772	87168	62775	2.89	7.96
	batch	4.6	-280			40765	3.50	
run 4	0	4.27	-280	2000		40765	3.00	7.61
	1	4.27	-230	2074	44388	40780	3.01	
	2	4.27	-231	2148	47730	43000	3.05	
	3	4.27	-235	2222	50823	43570	3.11	
	4	4.27	-230	2296	53693	43780	3.14	
	5	4.29	-230	2370	56364	44230	3.14	
	6	4.25	-215	2444	58856	44320	3.14	
	7	4.25	-210	2518	61186	44980	3.34	
	8	4.24	-215	2592	63370	44990	3.35	
	24	4.3	-215	3876	85885	67000	4.25	7.21
	25	4.31	-276	3950	86802	67908	4.29	
	26	4.31	-300	4024	87682	67990	4.29	
	27	4.35	-305	4098	88528	68790	4.32	
	28	4.35	-310	4172	89341	68342	4.32	
	29	4.35	-315	4246	90123	68645	4.32	
	30	4.35	-305	4320	90876	69564	4.35	
	48	4.45	-300	5772	100845	69560	4.45	7.01
	batch	4.45	-280			30120	5.10	

Table A 2.4 to be continued

run 5	0	4.45	-280	2000		30120	5.10	6.91
	1	4.45	-250	2074	33963	31200	5.50	
	2	4.45	-251	2148	37508	31435	5.60	
	3	4.45	-255	2222	40789	32570	5.71	
	4	4.45	-230	2296	43833	34890	5.72	
	5	4.45	-230	2370	46667	34657	5.72	
	6	4.45	-200	2444	49310	34990	5.72	
	7	4.45	-210	2518	51781	35456	5.72	
	8	4.44	-215	2592	54098	35786	5.72	
	24	4.53	-275	3876	77980	50564	7.02	6.92
	25	4.51	-276	3950	78953	50890	7.07	
	26	4.51	-245	4024	79886	51121	7.07	
	27	4.51	-205	4098	80783	51230	7.07	
	28	4.51	-210	4172	81646	51280	7.07	
	29	4.51	-215	4246	82475	51292	7.07	
	30	4.51	-205	4320	83274	51295	7.07	
	48	4.51	-300	5772	93849	51927	7.97	6.94
	batch	4.55	-340			30148		

Table A 2.5 Raw Data of Fed-Batch Experiments with CWP Containing 150 g l^{-1} Total Sugar

Time (h)	pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (%)	Biomass (g l^{-1})
				Control	Experiment		
run 1 0	4.55	-300	1000		35890	4.23	9.4
1	4.55	-310	1074	45549.69	40990	4.23	
2	4.55	-300	1148	53819.97	50567	4.25	
3	4.55	-321	1222	60980.5	50450	4.23	
4	4.55	-323	1296	67240.61	55000	4.26	
5	4.55	-300	1370	72760.08	60456	4.26	
6	4.55	-289	1444	77663.02	60345	4.23	
7	4.55	-290	1518	82047.26	60569	4.25	
8	4.55	-290	1592	85990.98	61890	4.23	
24	4.58	-290	2876	119214.4	100230	5.2	9.33
25	4.58	-299	2950	120334.4	100678	5.2	
26	4.58	-330	3024	121395.2	110456	5.2	
27	4.58	-320	3098	122401.6	115900	5.45	
28	4.58	-320	3172	123357.5	115980	5.45	
29	4.55	-320	3246	124266.6	120567	5.5	
30	4.55	-324	3320	125132.4	120450	5.5	
48	4.55	-330	4772	135773.3	123000	5.5	9.23
batch	4.6	-300	-			6.00	
run 2 0	4.6	-310	2000		52890	5.46	9
1	4.61	-312	2074	57023.41	53000	5.50	
2	4.61	-312	2148	60836.52	53789	5.51	
3	4.62	-300	2222	64365.18	53990	5.51	
4	4.62	-300	2296	67640.05	54600	5.51	
5	4.62	-300	2370	70687.59	54789	5.55	
6	4.63	-290	2444	73530.65	55460	5.55	
7	4.63	-299	2518	76189.16	55780	5.56	
8	4.64	-255	2592	78680.51	55000	5.45	
24	4.63	-325	3876	104368.3	96345	5.78	9.11
25	4.63	-300	3950	105414.6	96300	5.90	
26	4.63	-330	4024	106418.9	96900	5.70	
27	4.63	-330	4098	107383.6	97000	5.70	
28	4.66	-330	4172	108311.2	97230	5.65	
29	4.66	-330	4246	109203.6	98450	5.70	
30	4.66	-345	4320	110062.8	98450	5.68	
48	4.6	-356	5772	121436.7	70340	6.45	9.23

Table A 2.5to be continued

batch	4.64	-298	-				
run 3 0	4.64	-300	2000		50120	6.55	9.34
1	4.64	-300	2074	54199.88	50890	6.55	
2	4.64	-300	2148	57963.62	50990	6.55	
3	4.64	-320	2222	61446.57	53782	6.55	
4	4.64	-324	2296	64679.04	53860	6.55	
5	4.64	-325	2370	67687.11	54890	6.57	
6	4.63	-325	2444	70493.35	54900	6.60	
7	4.63	-325	2518	73117.43	55120	6.60	
8	4.64	-325	2592	75576.53	55129	6.60	
24	4.63	-340	3876	100931.6	80340	7.50	9.22
25	4.65	-340	3950	101964.4	82340	7.55	
26	4.65	-330	4024	102955.7	82560	7.55	
27	4.65	-330	4098	103907.9	82990	7.55	
28	4.65	-300	4172	104823.5	84560	7.66	
29	4.65	-300	4246	105704.3	86230	7.58	
30	4.65	-300	4320	106552.4	88990	7.45	
48	4.65	-356	5772	117779	60350	8.00	9.22
batch	4.65	-376				8.50	
run 4 0	4.65	-350	2000		40910	5.34	9.1
1	4.65	-345	2074	45629.56	41230	5.34	
2	4.65	-345	2148	49983.39	41290	5.39	
3	4.65	-335	2222	54012.43	41660	5.45	
4	4.68	-340	2296	57751.71	41900	5.45	
5	4.68	-345	2370	61231.4	43890	5.56	
6	4.68	-345	2444	64477.64	44000	5.60	
7	4.68	-340	2518	67513.14	44350	5.60	
8	4.68	-340	2592	70357.78	44550	5.60	
24	4.65	-340	3876	99688.25	60560	6.30	9
25	4.65	-330	3950	100882.9	63240	6.30	
26	4.65	-320	4024	102029.6	64670	6.30	
27	4.64	-320	4098	103131.2	65340	6.45	
28	4.63	-325	4172	104190.3	65400	6.55	
29	4.63	-325	4246	105209.2	65890	6.56	
30	4.63	-325	4320	106190.3	66000	6.67	
48	4.65	-378	5772	119177.1	70130	7.20	8.79
batch	4.65	-390				7.34	
run 5 0	4.65	-370	2000		42890	5.78	8.8
1	4.65	-345	2074	47314.99	43500	5.78	
2	4.65	-350	2148	51397.09	43670	5.78	

Table A 2.5 to be continued

3	4.66	-355	2222	55174.66	43500	5.78	
4	4.65	-350	2296	58680.56	44589	5.88	
5	4.64	-350	2370	61943.07	44900	5.89	
6	4.65	-350	2444	64986.7	45670	5.89	
7	4.64	-350	2518	67832.74	45897	5.89	
8	4.65	-350	2592	70499.84	45900	5.89	
24	4.67	-375	3876	97999.69	62130	6.12	8.7
25	4.67	-375	3950	99119.8	62300	6.12	
26	4.67	-375	4024	100194.9	64450	6.12	
27	4.67	-330	4098	101227.8	65230	6.34	
28	4.65	-325	4172	102220.7	65230	6.45	
29	4.65	-325	4246	103176.1	67000	6.55	
30	4.65	-335	4320	104095.9	65709	6.78	
48	4.65	-350	5772	116272.1	65890	6.78	8.55
batch	4.65	-370				7.50	

Table A 2.6 Raw Data of Fed-Batch Experiments with CWP Containing 200 g l^{-1} Total Sugar

Time (h)		pH	ORP	V(ml)	Sugar (mg l^{-1})		Ethanol (v v^{-1})	Biomass (g l^{-1})
					Control	Experiment		
run 1	0	4.55	-300	1000		28230	3.45	8.5
	1	4.55	-310	1074	42470	40234	3.50	
	2	4.55	-300	1148	54663	51023	3.70	
	3	4.55	-321	1222	65219	55,908	4.20	
	4	4.55	-323	1296	74448	66324	4.23	
	5	4.55	-300	1370	82585	73000	4.23	
	6	4.55	-289	1444	89813	80675	4.30	
	7	4.55	-290	1518	96276	87125	4.35	
	8	4.55	-290	1592	102090	94350	4.50	
	24	4.58	-290	2876	151068	132000	5.50	8.5
	25	4.58	-299	2950	152719	140890	5.60	
	26	4.58	-330	3024	154283	142090	5.65	
	27	4.58	-320	3098	155767	144340	5.65	
	28	4.58	-320	3172	157176	149000	5.6	
	29	4.55	-320	3246	158516	150560	5.6	
	30	4.55	-324	3320	159793	155500	5.6	
	48	4.55	-330	4772	175480	160345	6.2	8.4
	batch	4.6	-300	-		120900	6.20	
						69000	6.30	
run 2	0	4.6	-310	2000		69000	6.45	8.4
	1	4.61	-312	2074	74093	70000	6.40	
	2	4.61	-312	2148	78791	72023	6.30	
	3	4.62	-300	2222	83138	75321	6.30	
	4	4.62	-300	2296	87173	78450	6.20	
	5	4.62	-300	2370	90928	80450	6.25	
	6	4.63	-290	2444	94430	83020	6.30	
	7	4.63	-299	2518	97706	90120	6.30	
	8	4.64	-255	2592	100775	93250	6.30	
	24	4.63	-325	3876	132424	125000	6.60	8.1
	25	4.63	-300	3950	133713	128790	6.60	
	26	4.63	-330	4024	134951	129965	6.60	
	27	4.63	-330	4098	136139	132890	6.60	
	28	4.66	-330	4172	137282	133455	6.60	
	29	4.66	-330	4246	138382	134000	6.60	
	30	4.66	-345	4320	139440	136250	6.60	
	48	4.6	-356	5772	153453	138500	6.60	8

Table A 2.6 to be continued

batch	4.64	-298	-		100000	7.35	
					58345		
run 3 0	4.64	-300	2000		58345	6.10	8
1	4.64	-300	2074	63996	60250	6.60	
2	4.64	-300	2148	69210	62350	6.50	
3	4.64	-320	2222	74034	64689	6.30	
4	4.64	-324	2296	78511	66355	6.20	
5	4.64	-325	2370	82678	68450	6.10	
6	4.63	-325	2444	86565	73245	6.10	
7	4.63	-325	2518	90200	74350	6.10	
8	4.64	-325	2592	93606	75340	6.10	
24	4.63	-340	3876	128727	110450	6.10	7.9
25	4.65	-340	3950	130157	112350	6.10	
26	4.65	-330	4024	131530	115700	6.10	
27	4.65	-330	4098	132849	118560	6.10	
28	4.65	-300	4172	134118	121324	6.10	
29	4.65	-300	4246	135338	122090	6.10	
30	4.65	-300	4320	136512	126566	6.10	
48	4.65	-356	5772	152063	129700	6.10	7.8
batch	4.65	-376			92345	6.00	
					51250		
run 4 0	4.65	-350	2000		51250	6.00	7.7
1	4.65	-345	2074	56794	52345	6.10	
2	4.65	-345	2148	61908	55346	6.10	
3	4.65	-335	2222	66641	58346	6.10	
4	4.68	-340	2296	71033	62340	6.10	
5	4.68	-345	2370	75121	65450	6.10	
6	4.68	-345	2444	78934	67125	6.10	
7	4.68	-340	2518	82499	68345	6.10	
8	4.68	-340	2592	85841	68450	6.10	
24	4.65	-340	3876	120294	120234	6.50	5
25	4.65	-330	3950	121697	120345	6.50	
26	4.65	-320	4024	123044	121345	6.50	
27	4.64	-320	4098	124338	123338	6.50	
28	4.63	-325	4172	125582	123900	6.50	
29	4.63	-325	4246	126779	124350	6.50	
30	4.63	-325	4320	127932	125000	6.50	
48	4.65	-378	5772	143187	140350	6.50	3
batch	4.65	-390			120345	6.00	
					100250		

Table A 2.6 to be continued

run 5	0	4.65	-370	2000		100250	4.80	3
	1	4.65	-345	2074	104250	102500	4.80	
	2	4.65	-350	2148	107941	104356	4.80	
	3	4.66	-355	2222	111356	106000	4.80	
	4	4.65	-350	2296	114526	110345	5.10	
	5	4.64	-350	2370	117475	113250	5.10	
	6	4.65	-350	2444	120227	115345	5.10	
	7	4.64	-350	2518	122800	118340	5.10	
	8	4.65	-350	2592	125211	123500	5.10	
	24	4.67	-375	3876	150073	145350	5.10	3
	25	4.67	-375	3950	151085	147340	5.10	
	26	4.67	-375	4024	152057	148240	5.00	
	27	4.67	-330	4098	152991	150890	5.00	
	28	4.65	-325	4172	153889	151250	5.00	
	29	4.65	-325	4246	154752	153000	5.00	
	30	4.65	-335	4320	155584	154245	5.10	
	48	4.65	-350	5772	166592	155755	5.10	2.5
	batch	4.65	-370			141780	5.00	

A.3 Raw Data for Continuous Experiments

A. 3.1 Raw Data for the Variable Hydraulic Residence Time Experiments

Table A 3.1 Raw Data of Different Hydraulic Residence Time Eperiments

HRT (1/D) h	Percent sugar utilization	Effluent sugar (g l^{-1})	P (g l^{-1})	X (g l^{-1})	D*P (g $l^{-1}h^{-1}$)	D*X (g $l^{-1}h^{-1}$)	$Y_{x/s}$ (g g-1)	$Y_{p/s}$ (g g^{-1})	1/S (l g^{-1})
12.50	13.533	94.948	106.908	2.840	0.468		0.191	0.393	0.011
15.60	20.044	81.327	158.350	3.830	0.517	0.246	0.188	0.395	0.012
26.08	24.331	70.543	192.216	5.580		0.214		0.397	0.014
33.30	44.734	54.520	353.398	5.830	0.650	0.175	0.132	0.491	0.018
43.20	78.220	23.010	617.939	7.960	0.750	0.184	0.096	0.392	0.043
50.00	74.273	25.950	586.753	9.800	0.657	0.196	0.131	0.439	0.039
60.00	84.023	15.913	663.782	14.670	0.689		0.175	0.494	0.063

Table A 3.2 Raw Data of Specific Sugar Uitilziation Rate (q_s) and Specific Ethanol Formation Rate (q_p) at Different Hydraulic Residence Time Experiments

HRT (1/D) h	D (1 h^{-1})	q_s (gS g $X^{-1}h^{-1}$)	q_p (gP g $X^{-1}h^{-1}$)
12.50	0.080	0.419	0.165
15.60	0.064	0.341	0.135
26.08	0.038	0.156	0.093
33.30	0.030	0.227	0.111
43.20	0.023	0.240	0.094
50.00	0.020	0.153	0.067
60.00	0.017	0.095	0.047

A.3.2 Raw Data for Varaiable Feed Sugar Experiments

Table A 3.3 Raw Data of Different Feed Sugar Concentration Experiments

Feed sugar concentration (g l^{-1})	Percent sugar utilization	Effluent sugar (g l^{-1})	Etanol (v v^{-1})	D*P (gP $l^{-1}h^{-1}$)	X (g l^{-1})	D*X (g X l^{-1} h^{-1})	$Y_{p/s}$ (gP gS^{-1})	$Y_{x/s}$ (gX gS^{-1})	DS (gS $l^{-1}h^{-1}$)
55.06	71.62	15.63	2.32	0.34	4.86	0.09	0.46	0.12	0.73
102.92	57.58	43.66	3.70	0.54	4.80	0.09	0.49	0.08	1.09
124.16	47.07	65.71	3.65	0.53	4.71	0.09	0.49	0.08	1.08
148.35	28.24	106.45	2.05	0.30	3.55		0.39	0.08	0.77
177.28	27.47	128.58	2.03	0.30	3.90	0.07	0.33	0.08	0.90
199.30	26.58	146.33	2.00	0.29	3.34	0.06	0.30	0.06	0.98

A.4 Raw data of Packed Column Bio-reactor Experiments

A. 4.1 Raw Data for Variable Hydraulic Residence Times

Table A 4.1 Raw Data of pH and ORP at Different Column Heights

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
pH	5.25	4.24	4.36	4.36	4.37	4.38
ORP	-220	-250	-218	-219	-249	-272

Table A 4.2 Raw Data of Percent Sugar Utilization and Ethanol Concentration at Different Column Heights in Variable HRT Experiments.

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
HRT (h)	**Percent sugar utilization**					
64.43	0.00	0.61	0.63	0.65	0.69	0.69
49.78	0.00			0.64	0.67	0.70
37.3	0.00	0.59	0.60	0.61	0.68	0.68
28.44	0.00	0.58	0.62	0.62	0.65	0.66
22.45	0.00	0.50	0.59	0.58	0.60	0.65
17.57	0.00	0.43	0.47	0.52	0.58	0.63

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
HRT (h)	**P (g l^{-1})**					
64.43	0.00	17.38	17.70	17.78	17.93	18.01
49.78	0.00		14.22	15.80	18.17	19.59
37.3	0.00	14.77	14.69	15.41	16.83	17.06
28.44	0.00	14.46	14.62	15.09	15.33	15.41
22.45	0.00	9.80	10.51	10.59	10.90	11.61
17.57	0.00	8.69	10.27	10.27	10.59	10.27

Table A 4.3 Raw Data on Biomass Concentration at Different Column Heights in Variable HRT Experiments.

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
HRT (h)	**X (g l^{-1})**					
64.43		7.15	5.44	4.78	3.78	3.15
49.78		7.8	7.16	4.74	3.76	3.14
37.3		4	3.26	2.7	2.48	1.94
28.44		3.1	2.54	2.78	3.5	0.94
22.45		4.66	1.78	1.44	2.14	1.36
17.57		4.05	1.62	1.5	1.8	1.2

Table A 4.4 Raw Data for Effluent Sugar and Ethanol Concentrations, Productivity and the Yield Coeficient at Different HRTs.

	Effluent sugar concentration (g l^{-1})	**Effluent ethanol concentration (g l^{-1})**	**D*P (g P l^{-1} h^{-1})**	**$Y_{P/S}$ (gP gS^{-1})**
HRT (h)				
64.43	15.95	18.01	0.28	0.51
49.78	15.32	19.59	0.39	0.55
37.30	16.45	17.06	0.46	0.48
28.44	16.79	15.41	0.54	0.48
22.45	17.28	11.61	0.52	0.37
17.57	19.19	10.27	0.58	0.32

A. 4.2 Raw Data for Variable Feed Sugar Concentrations

Table A 4.5 Raw Data for Effluent Sugar Concentration and Biomass Concentration at Different Column Heights

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
Feed sugar concentration (g l^{-1})	**Effluent sugar, S (g l^{-1})**					
51.3		19.962	18.898	17.847	16.095	15.948
75.3		45.8	32.7	31.2	25.1	25.1
102.3		54.678	51.237	52.143	49.088	46.758
128.3		79.345	75.243	75.263	74.805	72.304
153.6		93.599	84.322	82.334	81.647	81.84
210.7		188.798	188.564	188.567	187.455	187.345

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
Feed sugar concentration (g l^{-1})	**Biomass, X (g l^{-1})**					
51.3	0	7.15	5.44	4.78	3.78	3.15
75.3	0	9.36	3.64	2.98	2.82	3
102.3	0	11.24	3.12	3.14	2.86	2.22
128.3	0	0.54	2.47	2.33	2.23	0.54
153.6	0	6.15	1.3	1.3	1.05	0.75
210.7	0	4.6	13.45	6.3	14.65	9.55

Table A 4.6 Raw Data on Variation of Ethanol Concentration with the Column Height

Height from the column inlet (cm)	**0**	**13**	**36**	**46**	**56**	**68**
Feed sugar concentration (g l^{-1})	**Ethanol, P (g l^{-1})**					
51.3	0	18.012	17.38	17.696	17.775	17.933
75.3	0	16.511	18.012	21.014	21.014	21.488
102.3	0	21.251	21.646	21.172	21.014	22.199
128.3	0	12.719	12.719	13.272	13.509	13.509
153.6	0	12.008	13.904	13.746	13.746	13.746
210.7	0	3.16	3.95	3.95	3.95	3.95

Table A 4.7 Raw Data for Effluent Sugar and Ethanol Concentrations, Productivity and Yield Coeeficient at Different Feed Sugar Concentrations.

Height from the column inlet (cm)	**Percent sugar utilization**	**Effluent sugar conc. ($g\ l^{-1}$)**	**Effluent Ethanol conc. ($g\ l^{-1}$)**	**$Y_{P/S}$ ($g\ P\ gS^{-1}$)**	**q_p ($gP\ gX^{-1}\ h^{-1}$)**
Feed sugar concentration ($g\ l^{-1}$)					
51.3	0.69	15.95	17.93	0.51	0.06
75.3	0.67	25.10	21.49	0.43	0.06
102.3	0.54	46.76	22.20	0.40	0.07
128.3	0.44	72.30	13.51	0.24	0.04
153.6	0.47	81.84	13.75	0.19	0.05
210.7	0.11	187.35	3.95	0.17	0.01