EFFECTS OF NITROGENOUS SUBSTANCES ON HEAT TRANSFER FOULING USING
MODEL THIN STILLAGE FLUIDS

BY

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THESIS

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Abstract

Fouling is unwanted deposition of materials on surfaces of processing equipment, which leads to additional investment, lower processing efficiency and potential fluid contamination. In the corn ethanol industry, fouling occurs when thin stillage is concentrated into condensed distillers solubles. Several researchers have investigated operating conditions and constituents' influence on fouling characteristics. However, understanding protein effects on fouling is limited despite its high concentration in thin stillage (17 to 33% db). Protein contributions to fouling have been investigated in the dairy industry. Whey proteins and calcium phosphate interact with each other or other proteins to form aggregates on heated surfaces. Maillard browning is another potential factor influencing fouling since amino acids in thin stillage are able to react with reducing sugars and form brown pigments. Proteins, their hydrolyzed products of amino acids and residual sugars in thin stillage contribute to fouling.

Due to complex components in commercial thin stillage, it is difficult to study a single effect on fouling without interference from other factors. The objective was to investigate effects of nitrogenous substances and protease on fouling using model and commercial thin stillage fluids. Nitrogenous substances urea and yeasts, as model protein sources in thin stillage, were mixed with glucose. Thermocouples in an annular probe were used to monitor surface temperature; fouling resistance was obtained by using overall heat transfer coefficients of fouled and unfouled surfaces. Fouling was characterized by maximum fouling resistance (R_{max}), induction period and fouling rate during 5 hr test periods.

Urea addition did not lead to fouling while glucose-yeast model fluids displayed fouling tendency that had a positive correlation with yeast protein concentration. Protease from pineapple stem (bromelain) incubation increased fouling in both model and commercial fluids, which were indicative that hydrolyzed molecules such as peptides, amino acids or protease itself can be involved in deposit formation. Adjustment of pH in model fluids during incubation reduced R_{max} and fouling rate and extended induction periods longer than 300 min. Fouling resistance profiles varied as the amount of bromelain changed from 1 to 3 g, showing a reduction in induction periods. Total suspended solids (TSS) of commercial thin stillage were measured during 14 days of storage; TSS was affected more by sample batch than storage time.

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CHAPTER 1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Due to requirements to reduce environmental pollution, ethanol has become a gasoline additive with increased production in US during the past two decades. The capacity of the fuel ethanol industry grew from 6.5 billion gallons in 2007 to 15.3 billion gallons in 2016 (RFA 2016). Compared with gasoline, ethanol contains more oxygen and higher octane, meeting the oxygen blending requirement by the US Clean Air Act (1990) (Tyner 2015). It reduces GHG emissions and decreases tailpipe emission of pollutants such as carbon monoxide, exhaust hydrocarbons and fine particulate matters.

Ethanol can be produced from various feedstocks such as corn, sugarcane, sorghum, cassava and cellulosics. The primary feedstock in US is corn. There are two major processes to produce ethanol from corn: wet milling (WM) and dry grind (DG) (Rausch and Belyea 2006). The former is capital and equipment intensive since corn kernels are fractionated into germ, fiber and starch before fermentation. DG ferments the whole kernel without fractionation with one primary coproduct: distillers dried grains with solubles (DDGS). This requires less capital investment. In 2016, 200 operating bioethanol plants produced 15.3 billion gallons and 90% of ethanol capacity came from DG (RFA 2016).

In dry grind processing, cleaned and ground corn is mixed with water to form a slurry. After cooking and liquefaction, starch is converted to short chain dextrins and oligosaccharides with α-amylase. Ethanol is produced during simultaneous saccharification and fermentation (SSF), and is recovered and purified by distillation columns and molecular sieves. The remaining unfermented mixture, whole stillage is centrifuged into thin stillage and wet cake. Evaporation concentrates thin stillage from total solids concentration of 4 to 6% up to 25 to 30%, which forms condensed distillers solubes (syrup) (Singh et al 1999). Condensed distillers solubes can be mixed with thin stillage and dried to make a commercial coproduct DDGS. To raise productivity and revenue in DG, several technique modifications have been applied or under study. New transgenic corn and GMO yeasts were used to reduce the cost of enzymes; the addition of granular starch hydrolyzing enzymes (GSHE) simplified the process and increased ethanol yield (Wang et al 2005). Postfermentation fraction and nutrient recovery add value to the

coproduct (Rausch and Belyea 2006). Energy investment is another major concern in dry grind processing. Meredith (2003b) reported 40 to 45% of thermal energy is used by evaporator and drying equipment. The occurrence of heat exchanger fouling during thin stillage evaporation decreases heat transfer rate and increases energy cost.

Fouling is defined as unwanted materials deposited or accumulated onto surfaces, which decreases heat transfer rates and leads to the loss of energy and contamination issues (Lalande et al 1989). Total fouling cost for industrialized countries can be over $4.4 billion annually and estimated loss due to heat exchanger fouling accounts for 0.25% of gross domestic product (GDP) of industrialized countries (Ibrahim 2012; Müller-Steinhagen et al 2005). One adverse effect caused by fouling is lower heat transfer efficiency. The gradually formed fouling layers resist transfer of heat and subsequently reduces outlet temperatures so that additional energy and surface area are needed to maintain a constant outlet temperature. Another detrimental effect is blockage of pipes or reduced cross sectional areas of tubes, which further increase pressure losses and pumping power requirements. CO_2 emissions, disposal of hazardous cleaning chemicals and localized deposit corrosion are other considerations of fouling. Although heat exchanger fouling problems have been studied since 1910, it is an unsolved problem in today's food, dairy, oil refinery and bioprocessing industries (Ibrahim 2012). Research on mechanisms of heat transfer fouling, effective methods to mitigate or avoid fouling and fouling deposition analysis are in progress. There are many factors, such as operating conditions, equipment design, and physical and chemical properties of fluids that impact heat transfer fouling. Operating conditions and equipment design involve bulk temperature, fluid velocity, viscosity and density, Reynolds number, pH, types and geometry of heat exchangers and material used for surfaces (Bansal and Chen 2006; Wilkins et al 2006b). Compared with physical properties, chemical properties of fluids varying with process sources can be more important, which include chemical composition, structures, thermal stability of components, compatibility in mixtures and chemical reactions during storage and processing (Ibrahim 2012). The combination of these factors complicates fouling procedures and makes study of heat transfer of fouling difficult. Fouling studies related in dairy industry are advanced (Bansal and Chen 2006; Sadeghinezhad et al 2015). β-Lactoglobulin has been verified as the dominant contributor to milk fouling (Bansal and Chen 2006; Sadeghinezhad et al 2015), which brings hope to develop practical methods to

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prevent fouling. However, research involving heat transfer fouling of thin stillage in corn ethanol is limited, especially with fluid component properties.

No studies have been focused on protein or glucose-protein effects on fouling characteristics of thin stillage in dry grind process despite relatively high protein content (17 to 33% db) (Rausch and Belyea 2006). Considering the complex and variable components of commercial thin stillage, model thin stillage fluids were established to simplify its composition.

1.2. Objectives

The purpose of this study was to investigate effects of nitrogenous substances and protease addition on heat transfer fouling properties using model and commercial thin stillage fluids. Nitrogenous substances (urea and yeast) were mixed with glucose to imitate commercial thin stillage.

1. Investigate fouling characteristics of glucose-urea and glucose-yeast fluids and explore concentration effects on fouling properties.
2. Observe effects of protease addition on fouling properties of glucose-yeast model fluids.
3. Compare fouling properties of commercial thin stillage with and without protease incubation; observe storage times impact on total suspended solids.

CHAPTER 2. LITERATURE REVIEW

2.1. Corn ethanol industry

Three commercial technologies are used to process corn: wet milling, dry grind and dry milling. Products from dry milling, such as flaking grits, smaller grits and meal, are mainly for human consumption and coproducts in animal diets (Rausch and Belyea 2006). Corn wet milling obtains highly purified starch through five main steps: steeping, germ recovery, fiber recovery, protein recovery and starch washing. Corn kernels are soaked in a weak sulfurous acid solution (2000 ppm as SO_2), that loosens starch granules from the endosperm matrix. A degermination disc mill grinds dewatered corn, which releases the germ; hydrocyclones separate germ from corn since the density of germ is lower than remaining corn components. Recovered germ is washed and dried. The underflow from germ separation is processed through mills and pressure fed screens, where fiber (pericarp and cell wall fiber) is washed and recovered. With addition of heavy steepwater (45 to 50% total solids), corn gluten feed is produced. A protein fraction is separated by centrifuge, concentrated and dried by a gluten thickener centrifuge, belt filter and dryer. The separated starch fraction passes through multistage starch washing hydrocyclones. Recovered starch is processed into ethanol and sweeteners such as corn, glucose and high fructose corn syrup (Galitsky et al 2003).

Despite relatively high valued products and coproducts, wet mills require higher capital investment and operating costs and usually are corporate owned. Therefore, 90% of fuel ethanol comes from corn dry grind, which ferments whole kernels without fractionation (RFA 2016). Corn transferred to the plants is graded for quality and cleaned. To reduce particle size, corn is ground by hammer mill or roller mill and mixed with water to form slurry. A jet cooker heats the slurry to 120°C with steam to sterilize and gelatinize starch (Singh and Johnston 2009). α-amylase enzyme breaks down 1,4-glucosidic linkages and converts starch into dextrins at 85°C during liquefaction (pH 5.4 to 5.8). Glucoamylase further breaks down carbohydrate chains (dextrins) and releases glucose, which is utilized by yeast to produce ethanol in the saccharification and fermentation tank at 32°C, pH 4.0 to 4.5 (Li et al 2014). Ethanol is concentrated to 190 proof (95%) by distillation, molecular sieves adsorb remaining water; a small amount of gasoline is added to make fuel ethanol (Rausch and Belyea 2006).

After ethanol removal, unfermentable material, called whole stillage, is centrifuged. The overflow stream, thin stillage, is evaporated to decrease moisture content and later combined with the underflow stream, wet cake, to produce distillers dried grains with solubles (DDGS) after drying (Fig. 2.1). Some thin stillage (15% or more) is recycled as process water for slurry preparation (Kwiatkowski et al 2006). DDGS contains remaining proteins, carbohydrates, oil and other nutrients and is used mainly in animal diets, especially ruminants.
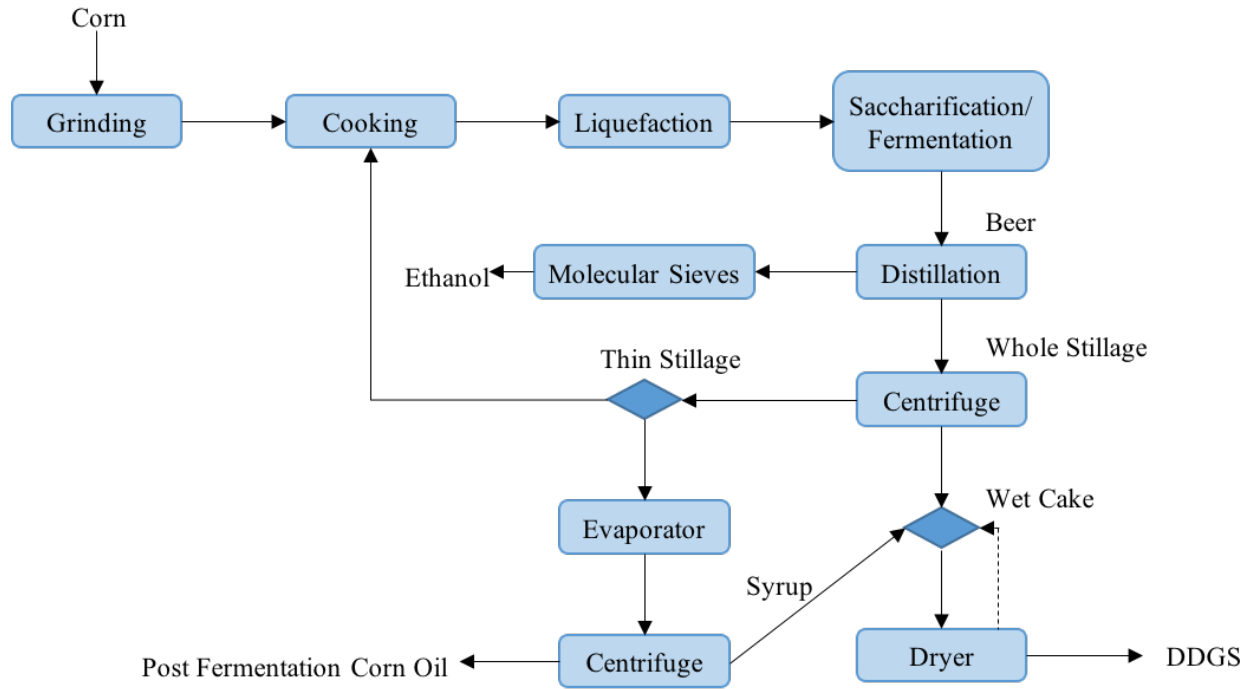
Figure 2.1. Dry grind process (Challa 2015).

2.2. Heat transfer fouling of evaporators

Fouling is defined as the deposition of unwanted materials on the surface of processing equipment including heat exchangers and boilers (Ibrahim 2012). It is a common phenomenon in food, dairy and corn ethanol industries when fluid is heated or pasteurized. The total cost for heat exchanger fouling accounted for 0.25% of highly industrialized countries' gross national product (GNP) in 1992 (Awad 2011). By 2012, the global heat exchanger market reached $12.7 billion with a 3 to 5% increase per year (Müller-Steinhagen et al 2011). Despite its sales volume, manufacturing is faced with increasing pressure to promote heat transfer efficiency and make heat exchangers more resilient to various process fluids. In a survey of 3000 heat exchangers from New Zealand, more than 90% of heat exchangers had fouling present (Müller-Steinhagen 2000). Problems related with heat exchanger fouling include loss of profits and environmental pollution. Adsorption and accumulation of deposits on heat exchanger surfaces reduce heat transfer rates and increase energy demands with longer manufacturing times. Frequently, when fouling becomes severe, manufacturing plants must shut down for cleaning, which leads to loss of revenue and additional operating costs. Repeated adhesion and removal of deposits make it possible to cause contamination in processing streams, affecting final product quality. To compensate increased heat transfer resistance caused by fouling, additional surface area is required during heat exchanger design, increasing capital costs (Shah and Sekulic 2003).

Two types of heat exchangers, shell and tube and plate heat exchangers are used widely in processing. The former consists of tubes surrounded by an outside shell (Fig. 2.2). One fluid flows within the tubes while another moves around the tubes within the shell. The shell and tube heat exchanger is a simple, effective and inexpensive configuration that is used for heating a liquid with steam or vapor (Blanchard 1992). Shell and tube heat exchangers are able to withstand high temperatures and pressures from vacuum to over 100 MPa (Shah and Sekulic 2003). The number of tubes are fixed after design; therefore, heat transfer capacities of tube and shell exchangers are less flexible than plate heat exchangers. A plate heat exchanger is made of plates which are clamped into a frame, forming channels for fluids to pass through (Fig. 2.3). The feasibility of adding and removing plates makes heat transfer capacity adjustable and easy for maintenance and deposit analysis. Despite low temperature gradient and excellent heat transfer behavior, the plate exchanger is more sensitive to fouling due to narrow channels and

joints between adjacent plates (Blanchard 1992). The plate heat exchanger is used mainly for liquid to liquid heat transfer, such as dairy, beverage, food processing and pharmaceutical industries (Shah and Sekulic 2003).
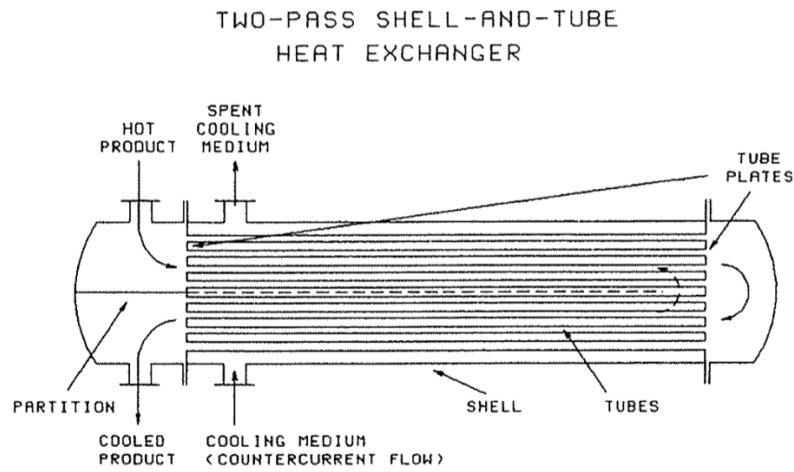
Figure 2.2. Sketch of shell and tube heat exchanger (Blanchard 1992).
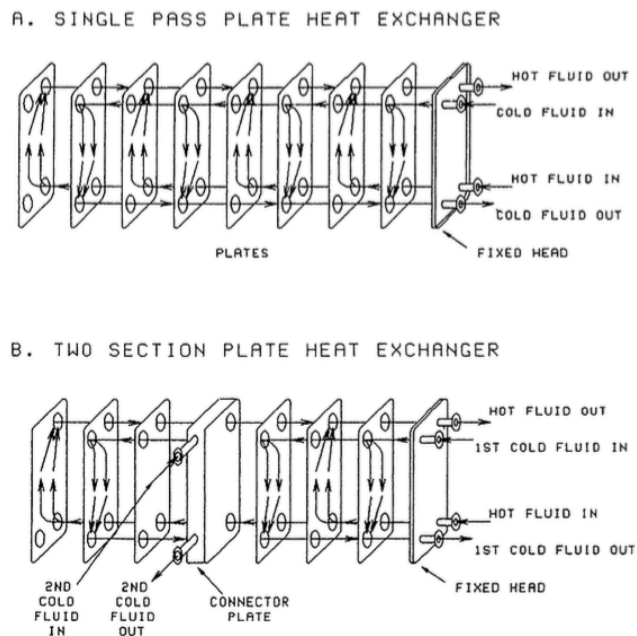
Figure 2.3. Sketch of plate heat exchanger (Blanchard 1992).

Besides design and geometrical effects of heat exchangers on fouling, surface properties of materials used, surface textures, surface energy, surface charge, existence of active sites and material already deposited also are associated with heat transfer fouling (Bansal and Chen 2006). Roughness can accelerate fouling by providing more effective surface area; hydrophobic areas are prone to adsorb more proteins (Wahlgren and Arnebrant 1991; Yoon and Lund 1994). Therefore, surface treatments such as coating and electro polishing are applied to reduce fouling by eliminating these factors (Kananeh and Peschel 2012).

To overcome fouling issues, other heating methods or heat exchangers with lower fouling resistance under specific conditions are being developed and used. They are fluidized bed heat exchangers, compact heat exchangers, heat exchangers with turbulence promoters, steam injection and steam infusion, microwave heating, ohmic heating and induction heating (Bansal and Chen 2006; Gough and Rogers 1987; Klaren 2003; Quarini 1995; Zaida et al 1986). In dry grind, falling film evaporators with vertical tubular heat surfaces are used widely to concentrate a solution due to high capacity, good heat transfer efficiency, short resident time and relatively easy maintenance (Monceaux and Kuehner 2009). The processing liquid is introduced at the top of tube bundles and distributed evenly by a distribution head such as perforated plates (Fig. 2.4). Liquid flows downward through heated tubes to form a film, where energy is transferred from shell side steam to tube side liquids by indirect contact. Vapor is generated on the tube side when the liquid temperature reaches to boiling point. Vapor, steam condensate and concentrate are collected at the bottom of the evaporator. Then vapor goes through a vapor separator to remove condensate and enter the next effect or to the condenser. For steam efficiency consideration, vapor compression (thermal or mechanical) and multiply effects are applied in the evaporator design so that vapor generated from previous effects can be used to heat liquid in next effect. Illustrated in Table 2.1, an example of energy savings using multiple effects in evaporators. Vapor is also used to preheat feed to raise its temperature close to boiling point before liquid enters tubes and therefore, evaporation occurs immediately after contact with heated surfaces (Blanchard 1992). Another energy saving design can be integration of first effect evaporator with distillation and molecular sieve (Meredith 2003a).
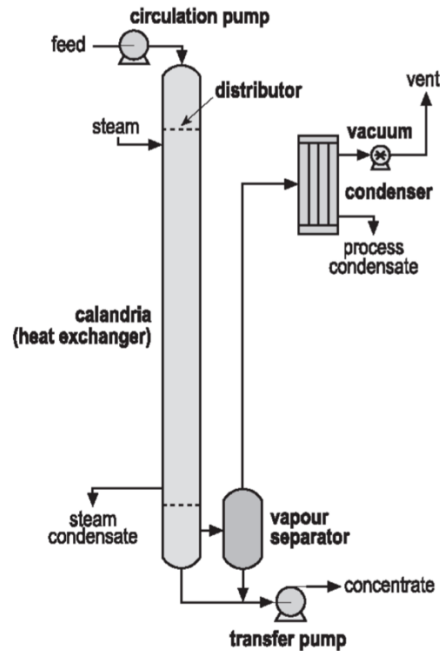
Figure 2.4. Single effect falling film evaporator (Monceaux and Kuehner 2009).

Table 2.1. Approximate steam consumption in typical multiple effect evaporators (Blanchard 1992).

	Lb. evap. /Lb. steam
Single effect	0.95
Double effect	1.75
Triple effect	2.50
Quadruple effect	3.25
Quintuple effect	4.20

Evaporator fouling is another issue that needs to be taken care of during thin stillage concentration. This issue generates additional spending on chemicals and energy usage and decreased yields due to plant shutdown. Concentration of processing fluids, degree of fouling and evaporator design determine cleaning times and intervals as well as amount of chemicals used (Meredith 2003a). To prevent and mitigate fouling, a typical dry grind plant cleans each effect of evaporators every week alternatively by caustic solutions soaking, water rinsing and

acid solution neutralization. The overall plant shutdown and cleaning occurs only once or twice a year.

Fouling is a complex phenomenon and it has been divided into five categories (Epstein 1983): crystallization, which is subdivided into precipitation fouling and solidification fouling, particulate, chemical reaction, corrosion and biological. Most of the time, fouling is a combination of two or more fouling modes. Thus, it is difficult to classify fouling into a single specific category. However, understanding these single fouling modes lays a foundation for realistic fouling studies.

Table 2.2. Classification of fouling mechanisms (Epstein 1983).

Fouling Category	Comments
Crystallization: Precipitation	Dissolved substances, such as salts, precipitate onto heat exchanger surfaces.
Crystallization: Solidification	Liquid or higher melting components such as paraffin wax freeze and crystallize on cooler surfaces.
Particulate	Insoluble solids in the processing fluids deposit on heat transfer surfaces.
Chemical reaction	Products of chemical reactions, rather than reactants, accumulate and deposit on heat transfer surfaces; common in petroleum refining, polymer production and food processing.
Corrosion	Corrosion substances deposit on heat exchanger surfaces.
Biological	Macro or microorganisms adhere to the heat transfer surface, grow and propagate.

2.3. Fouling in the dairy industry

Bansal and Chen (2006) comprehensively reviewed milk fouling in the dairy industry including fouling mechanisms and influential factors on milk fouling. Milk fouling can be caused by milk composition, operation conditions, microorganisms, heat exchanger types and fouling location. Type A fouling occurs when temperatures are between 75 and 110°C; type B fouling happens

when temperatures are more than 110°C (Changani et al 1997; Lund and Bixby 1975; Vietze et al 1998). Deposits formed at lower temperatures are soft, white and spongy with a high content of protein (50 to 70%), while deposits at higher temperatures are hard, gray and compact, and mainly composed of minerals (70 to 80%) (Bansal and Chen 2006).

Despite its relatively low content in milk total solids, β-Lactoglobulin (β-lg), one of the major whey proteins, was proposed as the dominant contributor to fouling (Bansal and Chen 2006; Itoh et al 1995; Visser and Jeurnink 1997). Whey protein leads to fouling of heat exchangers by first forming a protein monolayer on unheated or heated surfaces. At temperatures of 65 to 75°C, denatured whey protein, along with other proteins or calcium phosphate particles, aggregate in the bulk and transfer to the surface, interacting with the existed monolayer by diffusing through the layer or depositing onto the surface (Fig. 2.5). Removal of deposit and reentrainment with the fluid may occur repeatedly near the surface (Bansal and Chen 2006; Belmar-Beiny and Fryer 1993; Sadeghinezhad et al 2015; Visser and Jeurnink 1997). However, mechanisms and critical reactions causing fouling have yet to be determined. Some suggested protein denaturation as the main reaction while others suggested protein aggregation (Gotham et al 1992; Kessler and Beyer 1991; Lalande and Rene 1988). Some investigations demonstrated fouling was dependent on protein reactions only, while others stated role of mass transfer in fouling (Bansal and Chen 2006).
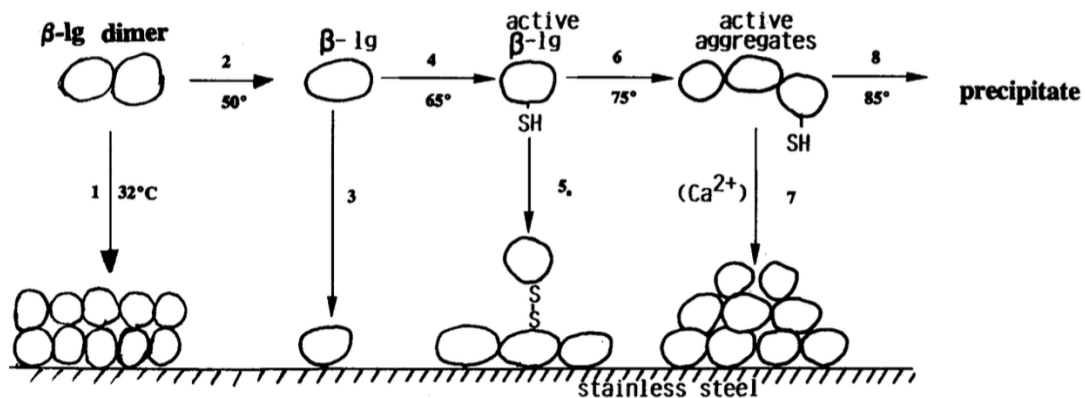
Figure 2.5. Dairy protein fouling mechanism on heat exchanger surface
(Visser and Jeurnink 1997).

Higher protein concentration leads to severe fouling, probably due to the structure and special groups of whey proteins (Bansal and Chen 2006; Belmar-Beiny and Fryer 1993; Burton 1967; Changani et al 1997). During denaturation, free and active sulphydryl groups are exposed when heated. These unfolded molecules are able to react with β-lg, other protein molecules or minerals, which promotes formation of protein aggregates. Formed complexes further transfer to the surface and adhere to it. Chemical modification of sulphydryl groups can reduce protein adsorption onto stainless steel surfaces, thus mitigating fouling (Itoh et al 1995). Calcium ions are able to enhance fouling by altering β-lg denaturation temperature and stability of casein micelles, attaching to β-lg and acting as bridges between aggregates and protein layers on the surface (Changani et al 1997; Christian et al 2002). Fung et al (1998) found that fat in milk did not cause fouling, but the damage to the fat globule membranes promoted fouling due to fusion of globules, which accelerated their movement towards surfaces (Fung et al 1998).

Apart from the influence of milk composition, heat exchanger fouling of milk also is related to operating conditions. Air existing in milk or formed by mechanical forces during the process can be nuclei for deposit formation, thus increasing fouling resistance (Jeurnink 1995). High fluid velocity and turbulence mitigate fouling with increased fluid shear stress. Absolute temperature and the temperature difference between bulk fluid and wall change the type or even determine the occurrence of fouling. Fluid pH affects three dimensional structure of β-lg and decreases electrostatic repulsion force among molecules at the isoelectric point (Visser and Jeurnink 1997).

Though much effort has been devoted to understand milk fouling, underlying mechanisms still are not clear. Variation of raw materials, complexity of the overall process and diversity of operating conditions challenge heat transfer fouling studies either in dairy or biofuel industries. We lack feasible technologies and methods to mitigate fouling or even completely eliminate it.

2.4. Fouling in the corn dry grind industry

Heat transfer fouling takes place in corn dry grind when thin stillage (5 to 10% total solids) is concentrated into condensed distillers solubles (30 to 50% total solids) by an evaporator. Due to high energy efficiency, short residence time, ability to hold large capacity of fluids and process heat sensitive and viscous liquids, multiple effect falling film evaporators are used in thin stillage

evaporation (Monceaux and Kuehner 2009). Multiple effect evaporators reduce energy consumption compared with single effect evaporators, making use of boiled water as vapor or heating steam to extract water from the next effect. For a dry grind plant with 100 million gal/yr ethanol yield, 270 tons of thin stillage/hr are produced; therefore, more than 2.0 million tons of thin stillage needs to be processed annually (Ingledew 2009). With considerable production of thin stillage and aggressive fouling during processing, it is necessary to investigate causes and properties of thin stillage fouling.

Though studies on thin stillage fouling are less reported than milk fouling, there are some valuable observations and conclusions in this particular field (Table 2.3). After separation of whole stillage, thin stillage (7 to 10% total solids) has a higher concentration of ash and oil but lower protein content compared to wet grains. Most proteins in thin stillage are water soluble. From amino acid analyses, glutamic acid accounts for highest concentration followed by leucine, which is in agreement with amino acid distributions of ground corn and yeast (Han and Liu 2010).

The annular fouling apparatus is able to imitate fluid conditions in industry and accelerate fouling within a short period of time. It has been used to monitor fouling resistance to find possible influential factors on fouling and develop optimal operating conditions as well as methods to reduce fouling. Wilkins et al (2006a) used an annular fouling apparatus to investigate repeatability of fouling rate and induction period of dry grind thin stillage, which was defined as time during which no fouling occurred. Fouling behaviors were correlated with the level of total solids and their composition, pH and Reynolds number (Re) (Wilkins et al 2006a; Wilkins et al 2006b). Increasing Re from 440 to 880 in the laminar flow region reduced fouling rates and increased induction times. Fouling deposits contained a higher amount of minerals than proteins, where the most abundant mineral was phosphorus. In a study on pH, Wilkins et al (2006b) discovered lower pH = 3.5 enhanced fouling which might be attributed to aggregation and precipitation of glucoamylase. Higher pH levels caused longer induction periods and increase of ash in deposits.

Challa et al (2015) investigated carbohydrates and their interaction effect on fouling. Pure starch model fluids led to more severe fouling compared with those containing glucose and short chain carbohydrates alone or their mixtures (corn syrup solids, glucose). Starch contributed to the fouling if it was left in thin stillage. Plant shutdown and cleaning extended the induction time of commercial thin stillage. Addition of 0.5 to 1.5% postfermentation oil (11 to 15% free fatty acid) had a higher fouling rate than that of commercial thin stillage; however, oil skimming during multiple effect evaporation did not decrease fouling (Challa et al 2017). But addition of refined corn oil (0.5, 1.0 and 1.5%) to thin stillage reduced fouling (Singh et al 1999).

Since interface temperature between heat exchanger surface and foulant is an important parameter to affect fouling behaviors in milk fouling (Burton 1968), Zhang et al (2017) studied initial temperature (T_i) and bulk temperature (T_b) effects on commercial and model thin stillage models (1% starch). Rapid fouling occurred in both commercial and model thin stillage samples when T_i =120°C and T_b = 80°C. High T_i increased fouling rates and R_{max} in both samples, while T_b had no impact on R_{max} of commercial thin stillage. Zhang et al (2017) also explored evaporator treatment effects on fouling by diluting concentrated thin stillage collected from different stages to the same total solids level and found evaporator effects did not alter fouling properties.

Membrane filtration has been proposed as a technique to mitigate fouling in dry grind process. Microfiltration (MF) and ultrafiltration (UF) was found to remove certain substances such as protein and fat from thin stillage due to membrane selectivity, which to some extent, reduced total solids concentration, leading to less fouling resistance. Agbisit et al (2003) compared surface fouling tendencies of light steepwater and membrane filtered light steepwater and pointed out that filtration would be a feasible technique to concentrate steepwater and increase heat transfer efficiency. Arora et al (2010) explored operating conditions for thin stillage filtration to achieve better permeate flux rates and discovered MF permeate displayed less fouling resistance compared with that of diluted thin stillage that contained the same solids content. Apart from differences in solids, variation of composition was related to fouling. Removal of fat and protein from thin stillage reduced fouling. Lipids can copolymerize proteins

and produce dark colored products deposited on the heat exchanger surfaces (Lund and Sandu 1981).

Table 2.3. Summary of previous studies on thin stillage fouling.

	Tb/Ti (°C)	Batch Volume (L)	Focus	Observations
Singh et al 1999	40/NA	30	refined corn oil	Fouling rate of wet milled thin stillage increased with added oil up to 1.41% wb. Fouling rate of dry grind thin stillage decreased with addition of oil (0.5 to 1.0% wb).
Agbisit et al 2003	40/99	30	microfiltration (0.1μm)	Microfiltration of steepwater reduced fouling tendencies and fluid solids content.
Wilkins et al 2006a	40/100	30	Total solids/Re	Thin stillage fouling increased with higher total solids; increasing Re from 440 to 880 decreased fouling.
Wilkins et al 2006b	40/100	30	pH/acid type	Thin stillage with pH 3.5 displayed shorter induction period and greater fouling rate with higher protein in deposit; acid type had no effect on fouling.
Arora et al 2010	60/100	30	microfiltration (0.1um)/dilution	Microfiltrated and diluted thin stillage showed less fouling; fouling reduction was greater in microfilterated sample.
Rausch et al 2013	50/100	50	starch/sucrose	Starch model fluids caused fouling; addition of starch to thin stillage increased fouling rate; sucrose model fluids did not foul.
Challa et al 2015	75/120	7	carbohydrate mixtures/starch type	Glucose and corn syrup mixtures or single compounds did not cause fouling; addition of glucose and corn syrup to starch models reduced fouling; high amylose and waxy starch gave different fouling profiles.
Challa et al 2015	75/120	7	postfermentation corn oil/glycerol/ solids	Concentrated thin stillage and thin stillage with extra oil (1.5%) or glycerol (1%) had increased fouling trends.
Zhang et al 2017	60,80/ 100,120	7	temperature/heat treatment/cleaning	T_i increased R_{max} and fouling rates of model and commercial thin stillage; fouling decreased after plant cleaning; heat treatment had no effect on fouling.

Wb: wet basis
T_i: initial probe temperature
R_{max}: maximum fouling resistance

Fouling studies are advanced in the dairy industry so that former experience and ideas can be introduced into corn processing researches; however, there are many differences between the two industries (Table 2.4 and Table 2.5). In dry grind, falling film evaporators with bundles of vertical tubes are used for heat sensitive thin stillage evaporation; while in the dairy industry, plate heat exchangers are mainly for milk pasteurization or ultra high temperature processing. The milk fluid temperatures are divided into two range with different deposit compositions; one is above 75°C (mostly in 95 to 110°C) and the other is above 120°C (Changani et al 1997). However, the operating temperature for thin stillage is lower, around 75°C. Typical pH of the thin stillage ranges from 3.7 to 4.7 while milk pH varies from 6.6 to 6.8 (Changani et al 1997; Wilkins et al 2006b). Composition difference between milk and thin stillage fluids is apparent.

Table 2.4. Operating conditions of milk and thin stillage.

Condition	Milk	Thin stillage
Heat exchangers	Plate heat exchanger	Falling film heat exchanger
pH	6.6 to 6.8[1]	3.7 to 4.7[2]
Bulk temperature	72°C; 135°C	75°C

[1] Changani et al 1997
[2] Wilkins et al 2006b

Table 2.5. Composition of milk and thin stillage.

Component	Milk (mean % wb)[1]	Thin stillage (% wb)
Moisture	87.5	92.9[2]
Total solids	13	7.1[2]
Proteins	3.4	1.13[3];1.53[4]
Minerals	0.8	0.68[4]
Fat	3.9	1.09[3]
Carbohydrate	4.8[6]	2.27[5]
Fiber	Not reported	0.09[3]

[1] Bansal and Chen 2006
[2] Rausch et al 2006
[3] Singh et al 1999
[4] Arora et al 2010
[5] Sum of Sugar profile, Singh et al 1999
[6] Represented by lactose %

Fouling is a complex process involving superposition of fouling modes and chemical reactions (Epstein 1983). Changes in operating conditions and fluid compositions exhibit diverse fouling behaviors, which helps determine influential factors on fouling and develop potential fouling mechanisms. Though whey proteins are not present in commercial thin stillage, other heat sensitive proteins or protein sulfhydryl groups in thin stillage make fouling studies on composition valuable.

2.5. Annular fouling probe

Fouling resistance can be measured by various methods. Thickness measurement and weighing of small deposits are two direct ways to monitor fouling but require high accuracy due to small changes; a disposable coupon, micrometer caliper or microscopy is usrful for thickness measurement (Awad 2011). An increase in pressure drop can be observed due to the smaller flow area in fouled regions or blockage of deposits. Therefore, the variation in pressure decrease verse time can reflect the degree of fouling and present an asymptotic shape that follows fouling (Awad 2011). Other nondestructive fouling assessments, such as laser techniques and radioactive tracers, help monitor deposit formation without terminating the experiment (Awad 2011). Thermal resistance monitors, such as annular probes, help obtain fouling resistance directly based on heat transfer theory.

Fetissoff (1982) constructed an annular fouling apparatus equipped with portable research fouling unit probe (PFRU). This apparatus was modified and used to study heat transfer fouling of olefin-kerosene mixtures, crude oils and autoxidation reactions of model indene solution (Asomaning and Watkinson 1992; Smith 2013; Wilson and Watkinson 1996).

With the design of PFRU, the annular fouling apparatus was used in thin stillage fouling studies due to its accuracy, portability and repeatable use (Agbisit et al 2003; Arora et al 2010; Challa et al 2017; Rausch et al 2013; Singh et al 1999; Wilkins et al 2006a; Wilkins et al 2006b). The annular fouling system consists of a concentric electrically heated stainless steel rod with an outer tube. A central rod is equipped with a resistance heater and four thermocouples, which are used to monitor inner wall temperature at different locations within the rod (Fig. 2.6). Regular thermocouple calibration increases accuracy of heat transfer measurement. Fluid passes over the

central rod within the outer tube wall and gradually forms deposits on the heated area. Degree of fouling is reflected by variation in surface temperature T_s (K), which is calculated by

$$T_s = T_w - \left(\frac{x}{k}\right)\frac{Q}{A}$$

T_w (K) is inner wall temperature measured by four thermocouples; Q (W) is power supplied to heater; A (m^2) is total surface area of probe and x/k (m^{2}K/kW) is distance of thermocouples to the surface divided by probe thermal conductivity. The overall heat transfer coefficient (U, W/m^{2}K) for the probe is:

$$U = \frac{Q/A}{(T_s - T_b)}$$

where T_b (K) is bulk temperature. Thermal resistance (R_f) is the reciprocal of overall heat transfer coefficient U, which is the sum of individual heat transfer resistances. Therefore, fouling resistance (R_f, m^{2}K/kW) can be obtained by subtracting the total thermal resistance at initial time t = 0 (clean surfaces) from thermal resistance at time t (fouled surfaces).

$$\frac{1}{U} = \sum heat\ transfer\ resistances$$

$$\frac{1}{U_{unfouled}} = R_i$$

$$\frac{1}{U_{fouled}} = R_i + R_f$$

$$R_f = \frac{1}{U_{fouled}} - \frac{1}{U_{unfouled}} = \frac{(T_s - T_b)}{Q/A} - \frac{(T_i - T_b)}{Q/A}$$

$U_{unfouled}$ is initial heat transfer coefficient with clean surfaces (t = 0)

R_i is initial heat transfer resistance

U_{fouled} is overall heat transfer coefficient with fouled surfaces at time t

R_f is heat transfer resistance from fouling

T_i is probe surface temperature at initial time (t=0)

T_s is probe surface temperature at time t

T_b is bulk temperature
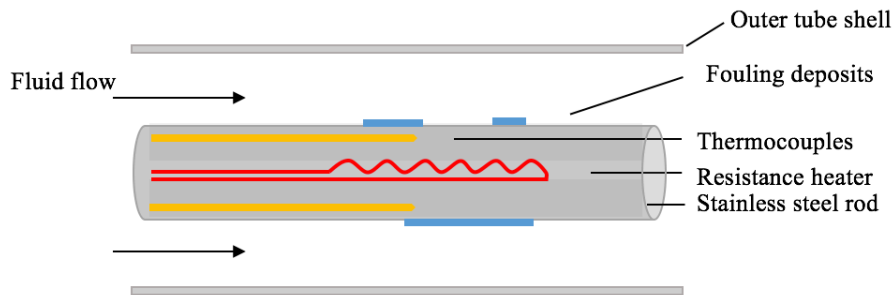
Figure 2.6. Annular fouling probe cross section.

The use of an annular fouling probe has some limitations. Though the deposit is visible and can be analyzed for further study, removal without damaging its microstructure and the probe surface is a challenge. Annular geometry makes it difficult to observe deposit structure and layers under microscopic devices due to surface curvature; heating section is limited to a part of the probe, thus producing a small amount of deposit; possible fluctuations in power supply may led to variation in surface temperature; generated deposits may cause pressure drop in the local area and promote convective heat transfer between solids and fluids with increased velocity (Epstein 1983). Compared with limited fouling data obtained by annular probe, a tube fouling unit heated directly by electrical current can be cut off to provide *in situ* analysis of deposits (Wilson and Watkinson 1996). A double pipe heat exchanger with a detachable window from the outer tube can allow morphological observations and weight measurement, which has been used to investigate fouling properties of milk with damaged fat globule membranes from 4 to 94°C using hot water as a heating medium (Fung et al 1998). Heat exchangers equipped with temperature monitors at inlet and outlet often are used to study milk fouling due to the same heat exchanger type in industry (Sadeghinezhad et al 2015). Methods and units chosen for fouling studies are based on research focuses, processing fluids and industrial operating facilities. Despite some drawbacks of annular fouling apparatus, it is a suitable testing system for lab scale fouling studies due to easy operation, local heat flux measurement, compact size and no heat loss to environment.

CHAPTER 3. EFFECTS OF NITROGENOUS SUBSTANCES ON HEAT TRANSFER FOULING PROPERTIES USING GLUCOSE-UREA AS A MODEL FLUID FOR THIN STILLAGE

3.1. Introduction

Proteins account for 8.1 to 11.5% db of the corn kernel and are distributed mainly in germ and endosperm (Ingledew 2009). In dry grind, yeast utilize few corn proteins in the form of amino acids during fermentation; therefore, most corn proteins remain in the unfermentable mixture called whole stillage. After centrifugation, most prolamins, such as zein, are removed from the liquid stream while water or salt water soluble albumins and globulins, exist in the thin stillage. Rausch and Belyea (2006) reported a higher protein concentration (20.1% db) in the thin stillage than fat, ash, fiber and other minerals. Crude protein content of commercial thin stillage varies with ethanol plants at different processing times, from 17 to 33% db (Arora 2009; Han and Liu 2010; Kim et al 2008; Rausch and Belyea 2006; Wilkins et al 2006a; Wilkins et al 2006b). From thin stillage fouling deposit analysis, Wilkins et al (2006b) found protein was the dominant component in deposits when pH decreased to 3.5 and protein concentration in deposits was 1.5 times that of thin stillage fluids. Elevation of drying air temperatures from 15 to 140°C decreased yield of extracted corn protein and loss of sulfhydryl groups, which implied corn protein can be denatured at a high temperature (Wall et al 1975). With these observations, proteins or broken down peptides, amino acids together with remaining carbohydrates in thin stillage are likely to cause heat transfer fouling during evaporation. However, study of protein effects on fouling is limited considering availability of purified corn protein, diverse types of protein with different physical and chemical properties in thin stillage and precise quantification.

Table 3.1. Crude protein content (% db) of commercial thin stillage from different references.

Component	Wilkins et al (2006a,b)	Rausch et al (2006)	Kim et al (2008)	Arora et al (2009)	Han et al (2010)
Total solids (%)	7.25	7.1	6.2	6.5	-
Crude protein (%)	16.8	33.4	21.0	23.5	20.4

In the dairy industry, heat exchanger fouling occurs when milk is pasteurized or under ultra high temperature processing. Milk fouling has been studied comprehensively and is related to whey protein adsorption, denaturation, aggregation and transfer during thermal processing (Fryer and Belmar-Beiny 1991; Visser and Jeurnink 1997). Composition of milk is simpler than that of thin stillage and has been well studied. Whey protein and casein are two major proteins, accounting for 0.44 and 2.6% wb of milk (Bansal and Chen 2006). Whey protein is recovered by membrane filtration and spray drying from whey, a coproduct solution mainly from cheese production (Tunick 2008). Whey protein is a market available product (35% protein) and water soluble within a wide pH range (2 to 10) (Tunick 2008). Therefore, milk model with alterable components has been created in fouling studies and avoided variations that existed in commercial processing streams (Changani et al 1997; Simmons et al 2007; Xin et al 2002). Market available β-lg promoted prediction of fouling mechanisms by working on potential influences from protein itself, such as adsorption capability, heat denaturation characteristics and interaction with other molecules (Itoh et al 1995; Visser and Jeurnink 1997). Removable heat exchanger plates make it possible to observe foulants structure under the scanning electronic microscopy; ex situ analysis by shakable water bath simplified heat exchanger geometry and fluids conditions, providing a basic idea of deposit formation and cleaning (Law et al 2009; Visser and Jeurnink 1997). Understanding of individual effects on fouling is the priority to explain the complicated fouling system and establish mathematic models to predict fouling process.

Since compositions of commercial thin stillage are variable and complex, model thin stillage fluids were made to investigate protein effects on heat transfer fouling. Urea was used to model protein sources due to lack of availability of pure corn protein ingredients. Based on work by Challa et al (2015), it was hypothesized that glucose combined with urea would have more rapid fouling characteristics than glucose alone. Urea is a commercial available chemical with high nitrogen concentration (46.7%) and good water solubility (108 g/100 ml water). Due to its N-H bonds, urea would behave similar to protein compounds found in thin stillage. The objective was to investigate heat transfer fouling properties of glucose-urea model fluids.

3.2. Materials and Methods

3.2.1. Sample preparation

Urea was purchased from Sigma Aldrich (St. Louis, MO). The amount of urea used was based on equivalent protein concentrations of thin stillage with 23 and 50% proteins dry basis (db). One concentration level mimicked protein content in commercial thin stillage and the other was an extreme level. A glucose (Sigma Aldrich, St. Louis, MO) model fluid without fouling was used as a benchmark to evaluate urea addition impacts on fouling properties. A 1% (w/v) starch model fluid with repeatable fouling resistance curves was used to ensure proper experimental manipulation and good condition of apparatus. By addition of glucose, total solids were maintained at 1 or 7%.

3.2.2. Fouling test

An annular fouling apparatus monitored fouling phenomenon, consisting of tank, pump, heat exchanger, flowmeter, annular probe and data logger (Fig 3.1). The portable annular fouling probe recorded probe inner wall temperature (T_w) and bulk temperature (T_b). Power was applied when fluid bulk temperature reached 79°C, which was heated by water bath through a heat exchanger. T_b was maintained at 80°C and initial probe temperature (T_i) was tuned at 120°C. Fouling tests were terminated when T_w reached 200°C or after 5 hr. Fluid viscosity and velocity were measured by viscometer at 75°C (model HBDVE, Brookfield Engineering Laboratories, Middleboro, MA, Spindle No.1, 60 rpm) and flowmeter. The flow rate and viscosity varied from 9.8 to 11.4 L/min with Reynolds number (Re) at 875±65 (Appendix C). Fouling resistance R_f (m^2K/kW) was obtained by following equations (detailed description see Chapter 2).

$$T_s = T_w - \left(\frac{x}{k}\right)\frac{Q}{A}$$

T_w (K) was inner wall temperature; T_s (K) was probe surface temperature; Q (W) was the power supplied to heater; A (0.004 m^2) was total surface area of probe and x/k (m^2K/kW) was distance of thermocouples to the surface divided by probe thermal conductivity, which were 0.0749, 0.1095, 0.0971 and 0.076 m^2K/kW. The overall heat transfer coefficient (U, W/m^2K) for the probe was:

$$U = \frac{Q/A}{(T_s - T_b)}$$

where T_b (K) was bulk temperature. Fouling resistance R_f (m^2K/kW) vs time was determined by overall heat transfer coefficients of fouled and unfouled surfaces (t = 0):

$$R_f = \frac{1}{U_{fouled}} - \frac{1}{U_{unfouled}}$$

The annular fouling probe apparatus was cleaned with detergent Alconox and tap water before fouling test (see appendix for detailed procedures). Fouling was characterized by fouling resistance R_f, maximum fouling resistance R_{max}, induction period (min) and fouling rate (m^2/kW/min). R_{max} represented maximum fouling resistance during the 5 hr test period. Induction period (IP) was defined as the period during which continuous moving average of three points was less than 0.05 m^2/kW. Overall fouling rate was determined as the slope of linear regression line of R_f vs time during 5 the hr period without fixed intercept except for starch and starch-urea model fluids (Fig. 3.2). Analysis of variance (ANOVA) and Tukey's honest significance test (R version 3.2.2) were used for fouling parameters. The statistical significant level was 5% ($p<0.05$).

Five treatments were used with two replicates per treatment (Table 3.2). Crude protein contents of model fluids were obtained by multiplying urea nitrogen % by protein factor 6.25 on dry basis.
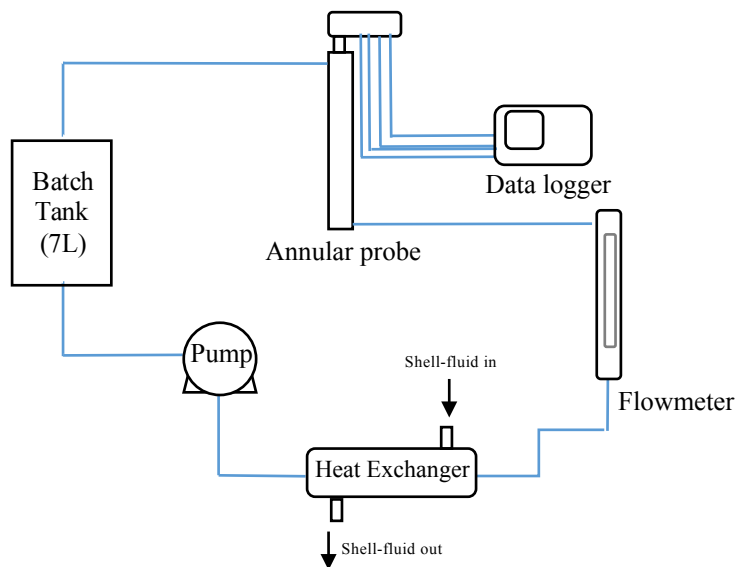
Figure 3.1. Fouling test apparatus.
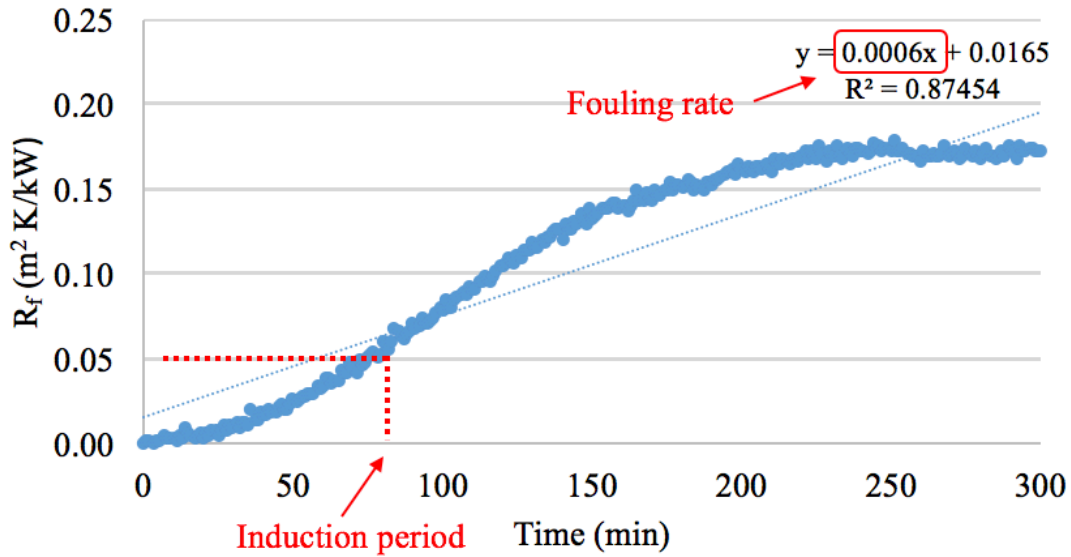
Figure 3.2. Definition of induction period and overall fouling rate.

Table 3.2. Glucose-urea and starch-urea model thin stillage fluids.

Treatment	Components	N % × 6.25 (db)	Total solids
GL	glucose	NA	7
GU23	glucose + urea	23	7
GU50	glucose + urea	50	7
STU	starch + urea	50	1
ST	starch	NA	1

Two replicates for each treatment.

3.3. Results and Discussion

Neither glucose nor urea-glucose mixtures displayed fouling behavior within the 5 hr test period (Fig. 3.3). Negative fouling resistance was observed in benchmark (GL) as had been reported in other fouling studies (Agbisit et al 2003; Arora et al 2010; Singh et al 1999; Wilson and Watkinson 1996). This may be due to disruption of the thermal boundary layer by particles, fluctuation of power supply or formation of rough deposits that facilitated heat transfer (Wilson and Watkinson 1996). The starch model fluid (ST) displayed rapid fouling phenomenon while GL gave a flat fouling resistance curve. Starch model fluid with urea (STU) had lower R_{max}

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compared to ST. For both ST and STU, fouling occurred rapidly within the first 50 min and then decreased by 25.2% and 33.2% respectively, with small fluctuations after reaching R_{max}. R_{max} of ST was 0.521 m^2K/kW, lower than reported by Zhang (2017) with the same T_i and T_b (0.71 m^2K/kW). Urea may have the ability to decrease fouling, which was reported from dairy fouling studies (Muir and Sweetsur 1976). Urea promoted heat stability of milk when heated at 120 and 140°C. Mean fouling rates and induction periods of STU and ST were similar (p<0.05) while R_{max} of STU was lower by 28.8% compared with ST (Table 3.3). There were variations in fouling resistance curves among treatment replicates (Fig. 3.4).
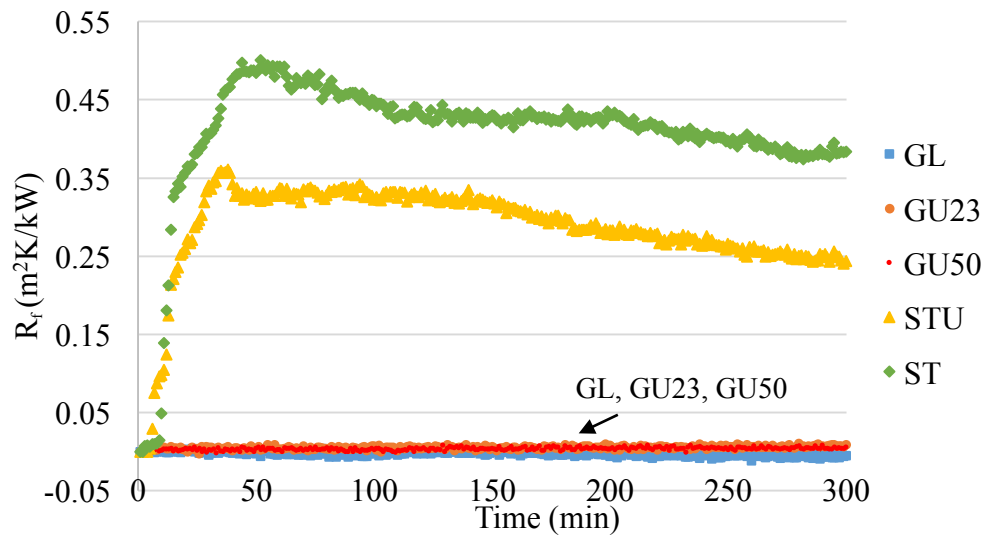
Figure 3.3. Mean fouling resistance of glucose-urea and starch-urea model thin stillage fluids. (Two replicates; GL: 7% glucose; GU23: glucose-urea mixture, 23% N×6.25; GU50: glucose-urea mixture, 50% N×6.25; STU: 1% starch-urea, 50% N×6.25; ST: 1% starch).

Table 3.3. Fouling properties of different thin stillage models[+].

Treatment	N % × 6.25 (db)	R_{max} (m^{2}K/kW)	Fouling rate×10^3 (m^{2}K/kW/min)	Induction period (min)
GL	NA	0.0115±0.0043a	<1.0	N/A
GU23	23	0.0260±0.0139a	<1.0	N/A
GU50	50	0.0237±0.0036a	<1.0	N/A
STU	50	0.371±0.039b	1.47±0.16a	6.0a
ST	NA	0.521±0.003c	2.00±0.42a	8.5a

[+] Means of two replicates, values with the same letter in each column are similar, p<0.05
GL: 7% glucose
GU23: glucose-urea mixture, 23% N×6.25
GU50: glucose-urea mixture, 50% N×6.25
STU: 1% starch-urea, 50% N×6.25
ST: 1% starch.
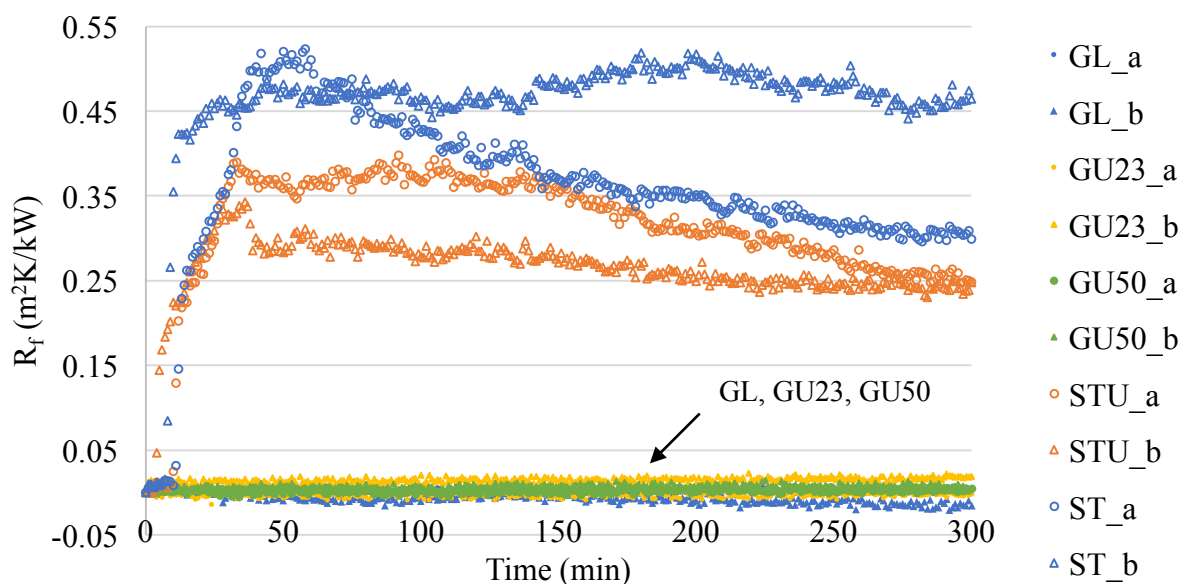
Figure 3.4. Fouling resistance of glucose-urea, starch-urea model thin stillage fluids. (GL: 7% glucose; GU23: glucose-urea mixture, 23% N×6.25; GU50: glucose-urea mixture, 50% N×6.25; STU: 1% starch-urea, 50% N×6.25; ST: 1% starch; lower case letters a, b represent replicates).

Urea addition to model thin stillage fluids did not accelerate fouling, which was beyond with previous hypothesis. Differences of chemical composition and structure between urea and proteins may explain that observation. Urea is a simple compound while proteins are macromolecules with three dimensional structures and sulfhydryl (-SH) groups that lead to fouling. In the dairy industry, proteins become unstable when heated and expose sulfydryl groups, which connect with other molecules by disulfide bonds and promote aggregate formation (Visser and Jeurnink 1997). The complexes transport to the surface and lead to fouling. Therefore, when choosing nitrogenous substances to replace thin stillage proteins, both chemical composition and structures should be taken into consideration.

3.4. Conclusions

A model fluid using glucose and urea did not display fouling within the 5 hr test period. Starch (ST) and starch-urea model fluids (STU) showed a rapid and severe fouling phenomenon while urea addition to starch fluids reduced fouling, reflected by decreased R_{max}.

CHAPTER 4. EFFECTS OF NITROGENOUS SUBSTANCES ON HEAT TRANSFER FOULING PROPERTIES USING GLUCOSE-YEAST AS A MODEL FLUID FOR THIN STILLAGE

4.1. Introduction

Based on information in the previous chapter, addition of urea to model thin stillage fluids did not lead to fouling. Looking for other suitable nitrogen sources similar to thin stillage proteins was the priority for later experiments. In simultaneous saccharification and fermentation (SSF), yeast (*Saccharomyces cerevisiae*) consume glucose and produce ethanol with the aid of glucoamylase. Ethanol is separated from beer by distillation and remaining unfermentable mixtures are centrifuged into wet grains and thin stillage. Most water and salt soluble proteins appear in the thin stillage, including yeast proteins that can have endogenous functional proteins such as enzymes, storage proteins and metabolized products. From the multiple linear regression model by Han and Liu (2010), yeast proteins accounted for 5% of proteins in intermediate products before fermentation due to the recycled thin stillage for slurries while they contributed 20% of proteins after fermentation. This observation can be explained partially by yeast growth and autolysis of yeast cells during or after fermentation. Yeast proteins in the form of free amino acids and soluble peptides were later recovered in thin stillage. During fermentation, yeast propagates during the log phase and produces ethanol at a faster rate, about 33 fold more than during the stationary phase. The number of yeast cells can increase from 6 to 10 million/ml at inoculation to 200 to 250 million/ml at the stationary phase, which indicates approximately 25 times more yeast will grow in the log phase period (Ingledew 2009). With increased cell numbers, the percentage of yeast components in the stream will increase. Yeast contain carbohydrate, protein, nucleic acids, lipids, inorganics and vitamins. Carbohydrates and proteins are the most abundant substances, which account for 18 to 44% and 38 to 59%, respectively, of the yeast. (Ingledew 1999; Ingledew et al 2009).

Considering increased yeast protein contribution to intermediate coproducts after fermentation and lack of an accessible commercially purified corn protein product, we used inactive yeast powder as a protein source to make model thin stillage fluids and speculated yeast protein could cause fouling. We hypothesized yeast protein or degraded peptides would interact with glucose during the fouling process and increase fouling over glucose or other carbohydrates alone. The

objective of this chapter was to explore fouling characteristics of glucose-yeast fluids and protein concentration impact on fouling properties.

4.2. Materials and Methods

4.2.1. Sample preparation

Inactive yeast powder purchased from Sigma Aldrich, St. Louis, MO was used as a major protein source for model thin stillage fluids. Inactivated yeast cells which were dried irrespective of enzyme activity, avoided potential biological activity during tests. The nitrogen concentration was measured by nitrogen analyzer (Elementar Rapid N Cube, Hanau, Germany) with combustion temperature at 950°C. Calibration was performed using aspartic acid to provide a correction factor. The average crude protein content of yeast powder was 47.5% (N% × 6.25). Crude protein contents of model fluids were obtained by multiplying yeast N% by 6.25 on dry basis (Table 4.1). Yeast powder was added to obtain fluids having 17, 23 and 28% crude protein concentrations (db). Glucose was added to maintain total solids of fluids at 7%. Considering potential reactions between glucose and yeast, pure yeast model fluids were made to compare fouling properties with those of glucose-yeast fluids. The same amount of pure yeast powder was dissolved with glucose and in tap water solution separately with total solids at 7 and 3.4%, respectively. Fouling resistance curves of model fluids were compared with two batches of commercial thin stillage, each batch was tested three times (TS1, TS2).

Table 4.1. Glucose-yeast (GY) and pure yeast (YP) model fluids with different crude protein levels.

Treatment	Components	N% × 6.25 (db)	Total solids
GY17	glucose + yeast	17	7
GY23	glucose + yeast	23	7
GY28	glucose + yeast	28	7
YP	yeast	47.5	3.4

Each treatment had two replicates.

4.2.2. Fouling test

The same annular fouling apparatus, cleaning protocols and operating conditions were used as described in Chapter 3. Viscosity was measured at 75°C and fluid velocity was recorded during fouling tests with Re = 1195 ± 60 (Appendix C). Fouling was characterized by using the same parameters listed in Chapter 3. Overall fouling rate was the slope of linear regression line of R_f vs time in 5 hr test while fouling rate after IP (after induction period) was the slope of regression line after induction periods within 5 hr (Fig. 4.1). All treatments in Table 4.1 with two replicates per treatment were randomized. Analysis of variance (ANOVA) and Tukey's honest significance test (R version 3.2.2) were used for fouling parameters. The statistical significant level was 5% (p<0.05). R^2 was the coefficient of determination, which reflected the linear relationship between two variables.
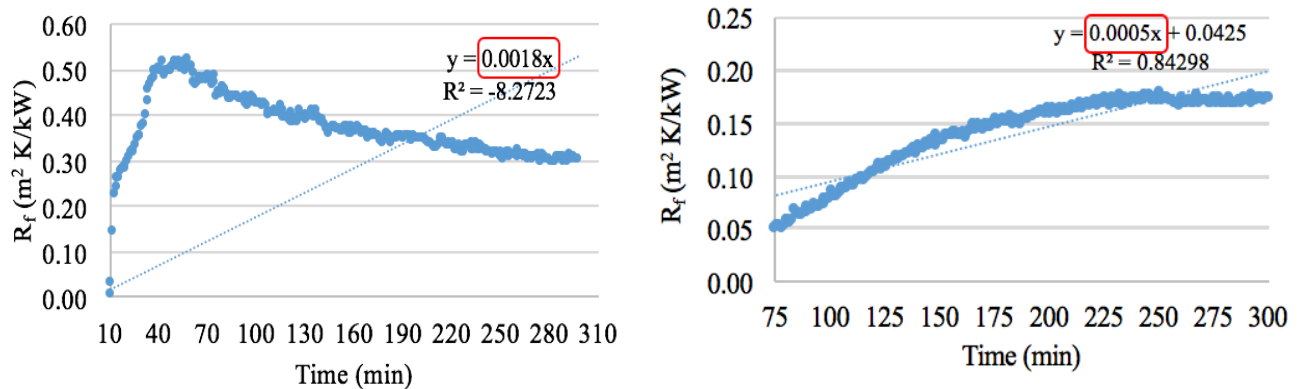
Figure 4.1. Definition of fouling rate after IP.
Left figure shows the linear regression line with an intercept at zero (for starch and starch-urea model fluids); right figure shows the linear regression line with a random intercept.

4.3. Results and Discussion

Two batches of commercial inactive yeast powders displayed different fouling curve profiles (Appendix D). To avoid variations from raw materials, adequate amounts of yeast powders were purchased for the following experiments.

For glucose-yeast model fluids, R_{max} and fouling rate increased while induction period time decreased with increasing crude protein concentrations. Mean R_{max} changed from 0.0922 m^2K/kW to 0.264 m^2K/kW while induction period shortened from 252 to 153 min (Table 4.2). There were no differences among R_{max}, induction period and fouling rates between GY17 and GY23. Increases in fouling properties were significant when fluid protein concentration increased from 23 to 28%. For all samples, fouling resistance increased with longer experimental time (Fig. 4.2). Fouling properties varied with protein concentrations with $R^2 = 0.88$ for R_{max} and overall fouling rate and $R^2 = 0.93$ for induction period. Fouling resistance curves of commercial thin stillage were almost linear with constant overall fouling rates while fouling resistance of glucose-yeast fluids were small during induction periods. For starch model fluids, fouling resistance increased to the maximum value within a short period of time followed by small fluctuations. In comparison with starch fluids, fouling resistance curve profiles of glucose-yeast fluids had general shapes similar to those of commercial thin stillage (Fig. 4.3). R_{max} and overall fouling rate of commercial thin stillage were similar to model fluids with 17 and 23% crude protein (Table 4.3). R_{max} and overall fouling rate of 1% starch model fluids were different from those of commercial samples. Due to smaller fouling resistance in commercial samples, the induction period of one sample batch was longer than 300 min according to its definition in methods. Therefore, induction period and fouling rate (after IP) of commercial thin stillage were not used for statistical analysis.

Table 4.2. Fouling properties of glucose-yeast model fluids and commercial thin stillage[+].

Treatment	R_{max} (5hr) (m^2K/kW)	Induction Period (min)	Fouling Rate $\times 10^3$ (5hr) $(m^2K/kW/min)$	Fouling Rate $\times 10^3$ (after IP) $(m^2K/kW/min)$
GY17	0.0922±0.0001a	252.0±7.1a	0.348±0.095a	0.888±0.061a
GY23	0.133±0.038a	220.5±13.4a	0.500±0.051a	1.01±0.355a
GY28	0.264±0.002b	152.5±7.8b	0.970±0.020b	1.50±0.126a

[+] Mean of two replicates, values with same letter in each column are similar, p<0.05.
For GY17, standard deviation is less than 0.0001.
GY: glucose yeast mixture with 17, 23 and 28% crude protein db.
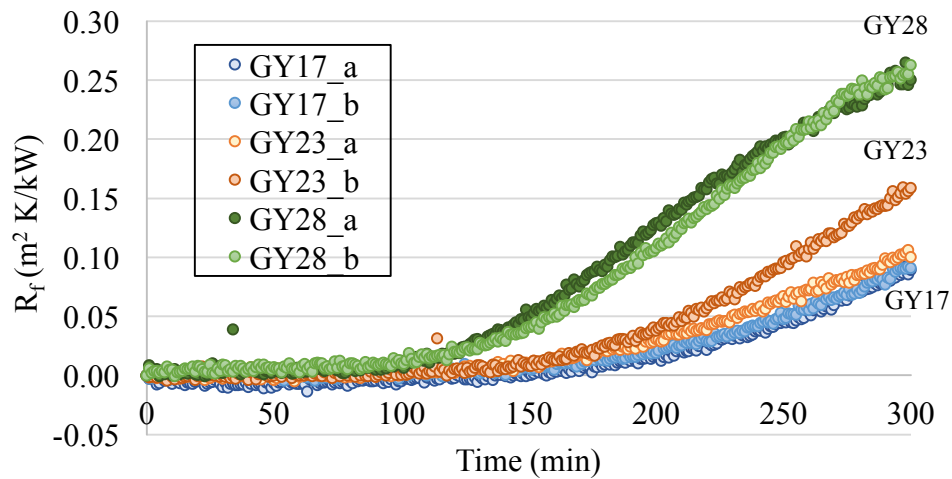
Figure 4.2. Fouling resistance of glucose-yeast model fluids at different protein levels. GY: glucose yeast mixture with 17, 23 and 28% crude protein db; lower case letters a, b represent replicates.

Table 4.3. Comparison of fouling properties between glucose-yeast model fluids, commercial thin stillage and starch model fluids[+].

Treatment	R_{max} (5hr) (m^2K/kW)	Induction Period (min)	Fouling Rate $\times 10^3$ (5hr) (m^2K/kW/min)	Fouling Rate $\times 10^3$ (after IP) (m^2K/kW/min)
GY17	0.0922±0.0001a	252.0±7.1a	0.348±0.095a	0.888±0.061a
GY23	0.133±0.038a	220.5±13.4a	0.500±0.051a	1.01±0.355ab
GY28	0.264±0.002b	152.5±7.8b	0.970±0.020a	1.50±0.126ac
TS	0.0533±0.0076a	N/A	0.284±0.183a	N/A
ST	0.521±0.003c	8.5±2.1c	2.00±0.42b	2.08±0.431bc

[+] Mean of two replicates, values with same letter in each column are similar, p<0.05.
For GY17, standard deviation is less than 0.0001.
GY: glucose yeast mixture with 17, 23 and 28% crude protein db; TS: thin stillage; ST: 1% starch model fluids.
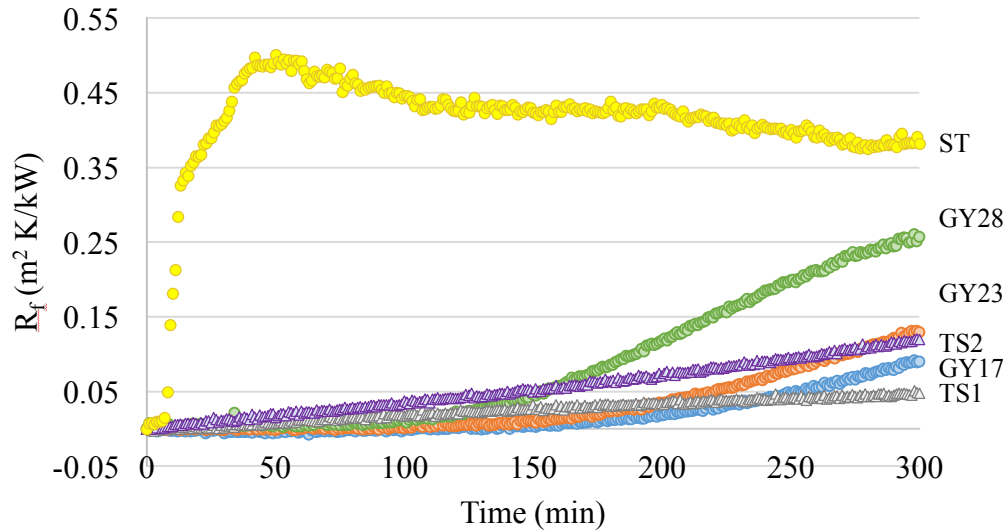
Figure 4.3. Fouling resistance of glucose-yeast, starch model fluids and commercial thin stillage. GY: glucose yeast mixture with 17, 23 and 28% crude protein db; TS: thin stillage (1,2 represent batch number); mean of three replicates for each batch of TS; mean of two replicates for GY, ST.

Pure yeast fluids (YP) displayed a higher fouling tendency than that of glucose-yeast fluids (GY) (Fig. 4.4). Even though YP had lower total solids (3.4%) than GY (7%), there was a difference in fouling rate between the pure yeast model ($0.0008 m^2 K/kW/min$) and yeast-glucose model ($0.0005 m^2 K/kW/min$), which indicated glucose solution was able to decrease the rate of deposit formation (Table 4.4). However, the reason is still not understood. Glucose-yeast model fluids gave repeatable fouling curves with small variations in fouling properties (CV < 30%). Therefore, glucose-yeast fluids can be used as model thin stillage fluids for fouling studies.
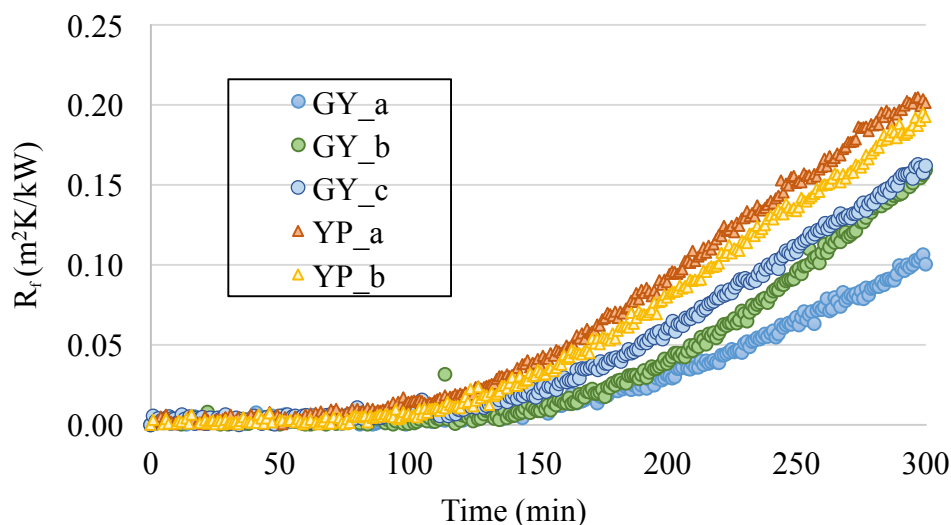
Figure 4.4. Fouling resistance of glucose-yeast and pure yeast model fluids.
GY: glucose and yeast mixture, 23% crude protein db; YP: yeast, 47.5% crude protein db; lower case letters represent replicates.

Table 4.4. Fouling properties of glucose-yeast and yeast model fluids[+].

Treatment	N% × 6.25 (db)	R_{max} (5hr) (m^2K/kW)	Induction Period (min)	Fouling Rate×10[3] (5hr) (m^2K/kW/min)	Fouling Rate×10[3] (after IP) (m^2K/kW/min)
YP	47.5	0.200±0.007a	167.0±5.7a	0.772±0.021a	1.15±0.02a
GY	23.0	0.142±0.032a	210.0±20.5a	0.510±0.041b	1.02±0.25a

[+] Mean of two replicates for YP (yeast) and three replicates for GY (glucose-yeast), values with same letter in each column are similar, $p < 0.05$.

4.4. Conclusions

A model fluid using glucose-yeast displayed repeatable fouling properties. Addition of inactive yeast powders accelerated fouling; there was a positive relationship between yeast protein concentration and fouling properties. R_{max} and fouling rate increased while induction time decreased when yeast concentration varied from 23 to 28%. Fouling phenomenon continued with longer testing time. Glucose solution may slow down fouling trend as reflected by diminished fouling rates.

CHAPTER 5. FOULING PROPERTIES OF GLUCOSE-YEAST MODEL FLUIDS WITH PROTEASE ADDITION

5.1. Introduction

Due to lack of available soluble corn protein from market and difficulty in purifying thin stillage proteins, glucose-yeast thin stillage fluid was used as a model to study protein fouling, especially for investigation of pretreatment effects on fouling. Proteases are enzymes which cleave specifically peptide bonds under optimal catalytic conditions. Addition of protease to glucose-yeast thin stillage fluid will hydrolyze yeast proteins.

Various proteases have been used in corn processing for different purposes. For instance, GC212 from DuPont Industrial BioSciences was added with GSH (granular starch hydrolyzing enzyme) in E-milling to loosen protein matrix of ground corn slurries; NS50045 from Novozymes was used to release free amino nitrogen (FAN) from germ, which was later consumed by yeast as a nutrition supplement (Li et al 2014; Vidal et al 2011). Johnston et al (2004) studied effects of soaking, grinding and enzyme incubation steps on quality and yields of recovered products such as germ, starch, fiber and gluten. Addition of bromelain achieved similar starch yields as that from traditional procedures where SO_2 was used. Trypsin and papain were used to remove remaining protein in high amylose (57%) corn starch obtained by lab scale wet milling (Vojnovich et al 1960). In general, protease use during corn processing was to weaken the protein matrix, promote separation and increase coproduct levels.

Stem Bromelain (EC 3.4.22.32) is a cysteine endopeptidase from pineapple stems. It is able to maintain activity in a broad pH range with an optimal condition near neutral pH. Thiol groups in the active site facilitate peptide breakdown and release of amine terminus. Bromelain is able to degrade a wide range of proteins, including glutelin matrix around starch and other classes of proteins in corn kernels. From sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) data, it was determined that corn proteins were broken down into peptides with molecular mass around or below 10kDa after enzymatic treatment (Singh and Johnston 2004). Z-Arg-Arg-NHMec usually is used as a substrate to measure bromelain enzymatic activity based on fluorescent intensity of hydrolyzed products (Rowan and Buttle 1994; Rowan et al 1990). Though it belongs to the papain family, stem bromelain displayed distinctive catalytic and

inhibition behaviors compared with other members. For instance, it showed resistance to inactivation by chicken cystatin and need of Arg in both P1 and P2 sites of substrates for efficient cleavage (Rowan and Buttle 1994). Stem bromelain has been reported to cleave arginine-alanine and alanine-glutamic acid bonds of glucagon and display preference for glutamic acid, aspartic acid, lysine or arginine in the P1 site of substrates (Arshad et al 2014). Positional scanning synthetic combinatorial libraries (PS-SCLs) found its catalytic function performs at the site of arginine (Choe et al 2006). Considering the availability and price of enzymes, bromelain was used to study protease addition effects on model thin stillage fluids. It was hypothesized that degraded yeast protein would decrease fouling due to smaller molecular sizes, unfolded structures and decreased water solubility.

5.2. Materials and Methods

5.2.1. Protease incubation

Inactive yeast powder (Sigma Aldrich, St. Louis, MO) was dissolved in 900 ml water to make a slurry (water : solids = 3.79:1). Blank samples were used as controls for protease pretreatment. First, pH value was adjusted to 5.00±0.10 by H_2SO_4 (10N) and NaOH (0.1 mol/L). Since acid type for pH adjustment did not affect fouling characteristics as reported by Wilkins et al (2006b), sulfuric acid was used. Bromelain (Sigma Aldrich, >= 3.0 units/mg, >= 35% biuret) was added in specific amounts (1, 2 or 3 g) to Erlenmeyer flasks and mixed with yeast slurries thoroughly using a stainless steel lab spoon. For enzymatic incubation, slurries were put into 48°C water bath and shaken at 50 rpm for 6 hr. Subsamples (20 ml) were taken from blank and treatment slurries after 6 hr. To inactivate enzyme activities, remaining mixtures were heated to 70°C for 10 min, then cooled and stored at room temperature for fouling tests within 72 hr. Trichoroacetic acid (TCA) was used to terminate the enzymatic reaction. Ten ml subsamples were placed into test tubes and TCA (20% w/v trichoroacetic acid, Sigma Aldrich, St. Louis, MO) solution was spiked into subsamples by 1:1 volume. TCA can precipitate protein by disrupting hydrogen bonds of water shells around protein or damaging protein folded structures (Lorsch 2014). Some peptides can become insoluble at higher TCA concentrations from 2 to 12% while others remain soluble in that concentration range. It was hypothesized that 10% TCA could solubilize peptides with 3 to 4 amino acid residues (330 to 380 Daltons) (Yvon et al 1989). The solution was mixed thoroughly by shaking. After mixing, samples were cooled to 4°C for 10

min and centrifuged at 3500×*g* for 10 min. Supernatants were discarded; pellets were washed by 10 ml cold TCA solution (10% w/v) and centrifuged at at 3500×*g* for 10 min. After centrifugation, supernatants were discarded and pellets were stored at 4°C for crude protein analysis, which was measured by nitrogen analyzer (Elementar Rapid N Cube, Hanau, Germany) using 250 mg standard method with combustion temperature at 950°C.

5.2.2. Sample preparation

To maintain total solids at 7%, glucose was added (252.5±0.5g) to incubated yeast slurries for fouling tests. Five treatments (7 L) were conducted and each was replicated three times. They were initial glucose-yeast fluids without incubation procedures and protease (GY_Initial), blank fluids with incubation procedures but without protease (GY_Blank), treatment samples with 1, 2 or 3 g protease (Table 5.1, Fig. 5.1). For yeast slurries (900 ml) with pH adjustment, pH (Waterproof pH 150, Oakton, Vernon Hills, IL) was maintained within the range of 5.00±0.10 during incubation, which was an optimum value recommended in E-milling (Johnston and Singh 2004). The pH values of original yeast slurries were 5.55 to 5.70. Fouling tests with pH adjusted or preheated yeast slurries also were conducted in order to determine incubation conditions' impact on heat transfer fouling. Since heat incubation time did not affect fouling properties, it was shortened to 2 hr. Measurement of pH with these model thin stillage fluids were conducted before fouling test (Fig. 5.2). Two batches of commercial samples were used to compare fouling characteristics with those of protease treated model fluids.

Table 5.1. Description of treatments using protease, pH adjustment and incubation.

Treatment	Composition (23% equivalent protein on db)	Protease (g)	pH adjustment	Incubation Time (hr)
GYE1	glucose, yeast	1	Yes	6
GYE2	glucose, yeast	2	Yes	6
GYE3	glucose, yeast	3	Yes	6
GY_Blank	glucose, yeast	NA	Yes	6
GY_Initial	glucose, yeast	NA	No	NA

Each treatment had three replicates.
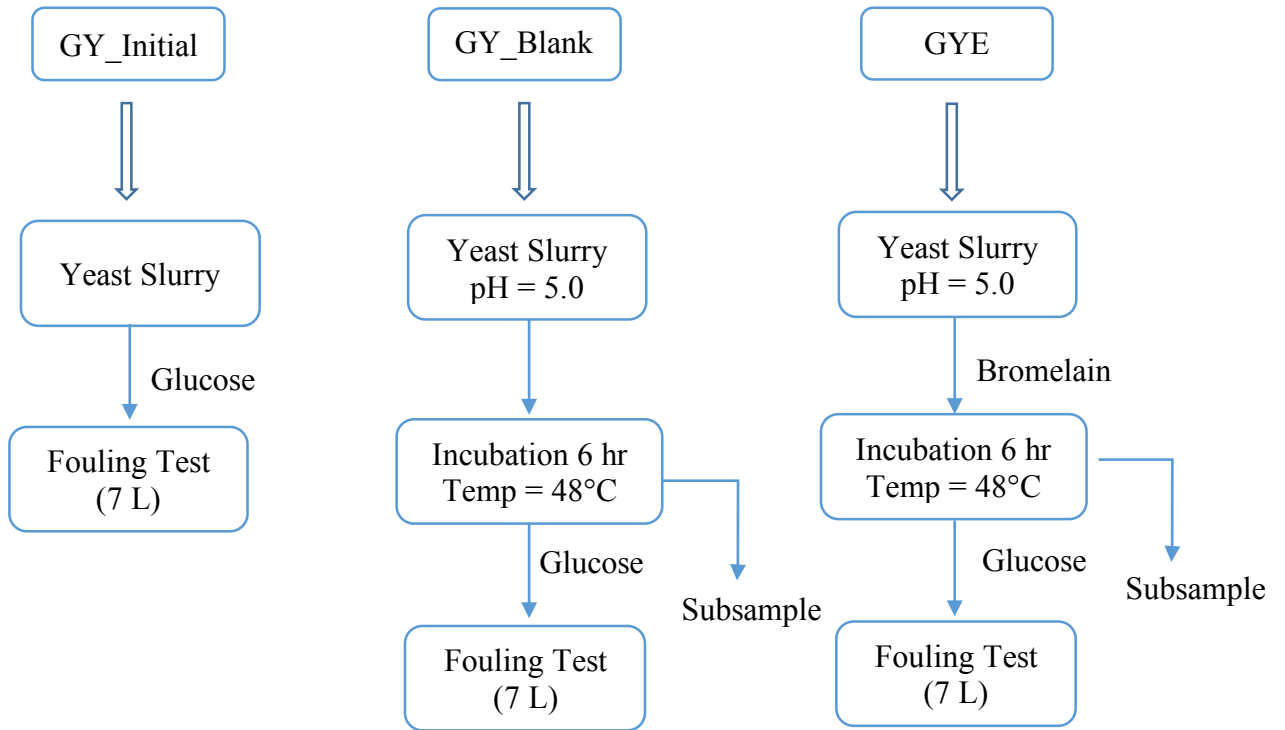
Figure 5.1. Flowchart of different treatments.
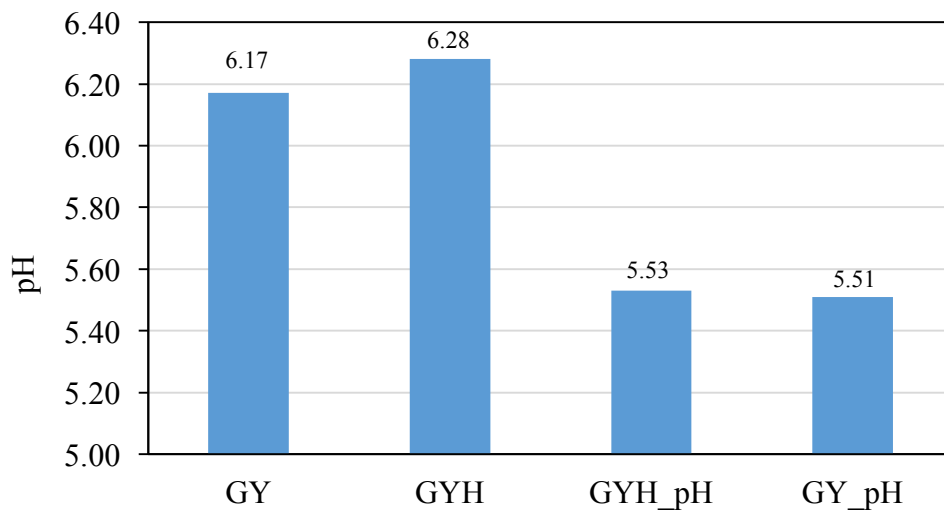
Figure 5.2. pH values of glucose-yeast thin stillage fluids before fouling test (7 L).
GY: glucose-yeast model fluids; H: 2 hr heating; pH: yeast slurry with pH adjustment; each value is mean of two replicates.

5.2.3. Fouling test

The same annular fouling apparatus, cleaning protocols and operating conditions were used as described in Chapter 3. Viscosity and fluid velocity were measured at 75°C during fouling tests with Re = 1215±40 (Appendix C). Fouling was characterized by using the same parameters in Chapter 3. Overall fouling rate was the slope of linear regression line of R_f vs time in 5 hr test while fouling rate after IP (after induction period) was the slope of regression line after induction periods within 5 hr (Fig. 5.3). Analysis of variance (ANOVA) and Tukey's honest significance test (R version 3.2.2) were used for fouling parameters. The statistical significant level was 5% (p<0.05).
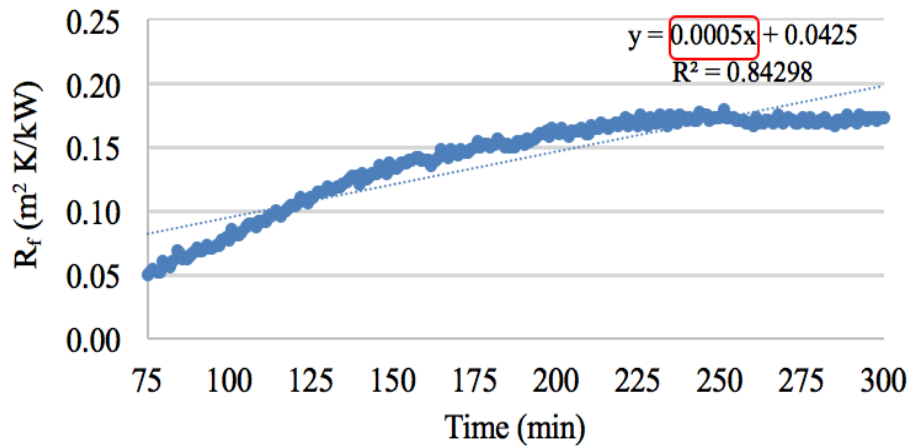
Figure 5.3. Definition of fouling rate after IP.

5.3. Results and Discussion

All treatments with protease showed fouling phenomenon. More deposits were formed with a longer test time. The coefficients of variation (CV) for R_{max}, induction period and overall fouling rate were less than 20%. For protease treated fluids, fouling occurred more rapidly with higher amounts of protease, which was reflected by shortened induction periods (Table 5.2 and Fig. 5.4). Though there were no differences in R_{max} and overall fouling rate among three protease treated fluids; fouling resistance curves shapes were different. Glucose-yeast fluids with 1 and 2 g protease addition, as well as initial fluids resulted in almost linear curves ($R^2 > 0.98$) especially after induction time. Fluids with 3 g protease had shorter induction periods at the beginning and plateaus after 250 min (Fig. 5.5). Fouling rates after induction periods were similar between GYE1 and GYE2 (Fig. 5.6). However, GYE1 had a higher fouling rate after IP than overall

fouling rate. There were no differences between overall fouling rates and fouling rates after IP in GYE2 and GYE3.

Table 5.2. Fouling properties of glucose-yeast models with and without protease incubation[+].

Treatment	R_{max} (5 hr) (m^2K/kW)	Induction Period (min)	Fouling Rate$\times 10^3$ (5 hr) ($m^2K/kW/min$)	Fouling Rate$\times 10^3$ (after IP) ($m^2K/kW/min$)
GYE1	0.165±0.002a	154.7±10.8a	0.623±0.015ab	0.772±0.047ab
GYE2	0.199±0.023a	119.0±9.5b	0.687±0.050a	0.863±0.105a
GYE3	0.188±0.008a	76.0±2.6c	0.606±0.048ab	0.573±0.047b
GY_Blank	0.0236±0.0233b	>300d	<0.1c	<0.1d
GY_Initial	0.156±0.025a	95.0±7.8c	0.510±0.094b	0.504±0.119b

[+] Means of three replicates, values with same letter in each column are similar, $p<0.05$.
IP: induction period
GYE: glucose-yeast fluids with protease addition (1, 2, 3 g)
GY_Blank: glucose-yeast fluids with incubation procedures but without protease
GY_Initial: initial glucose-yeast fluids with no protease and incubation
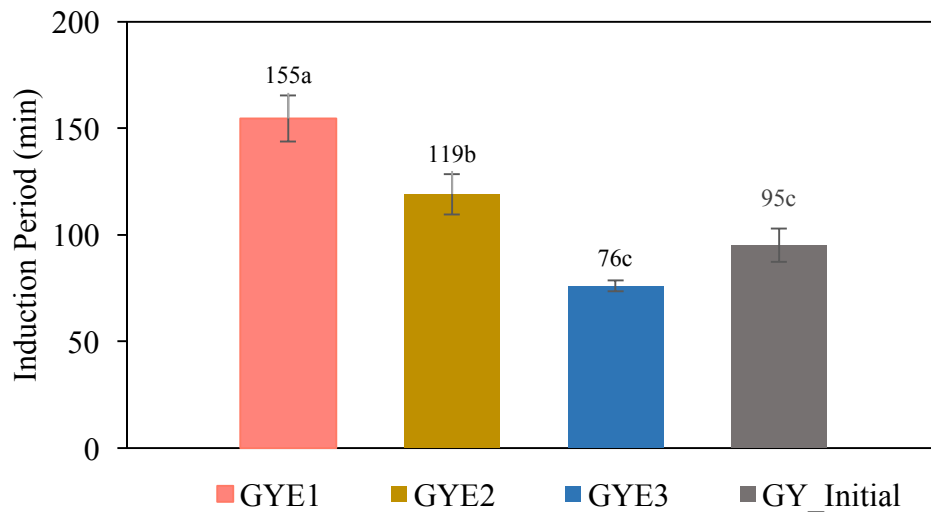
Figure 5.4. Mean induction periods of glucose-yeast thin stillage fluids with and without protease.
GYE: glucose-yeast fluids with protease addition (1, 2, 3 g); GY_Initial: initial glucose-yeast fluids with no protease and incubation; means of three replicates. Error bars represent standard deviation. IP of GY_Blank was longer than 300 min (not shown).
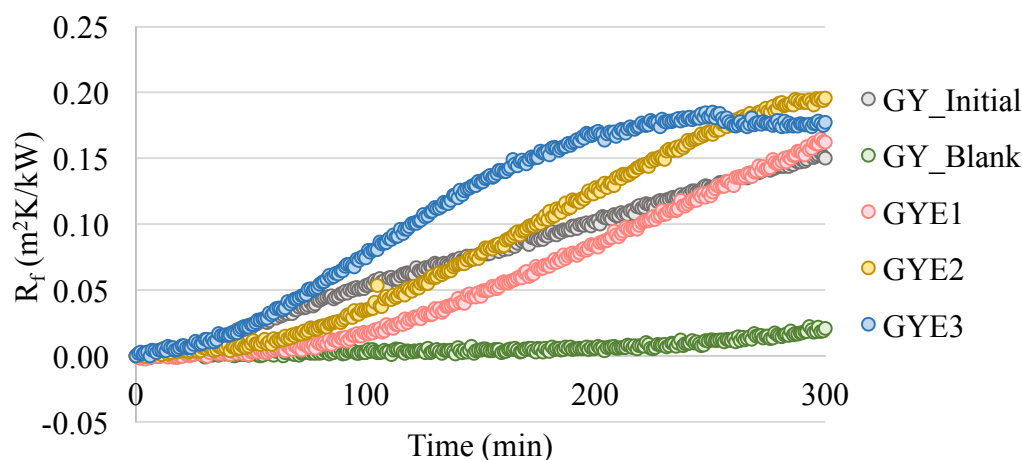
Figure 5.5. Mean fouling resistance curves of glucose-yeast thin stillage fluids with and without protease.
GYE: glucose-yeast fluids with protease addition (1, 2, 3 g); GY_Blank: glucose-yeast fluids with incubation procedures but without protease; GY_Initial: initial glucose-yeast fluids with no protease and incubation; means of three replicates.
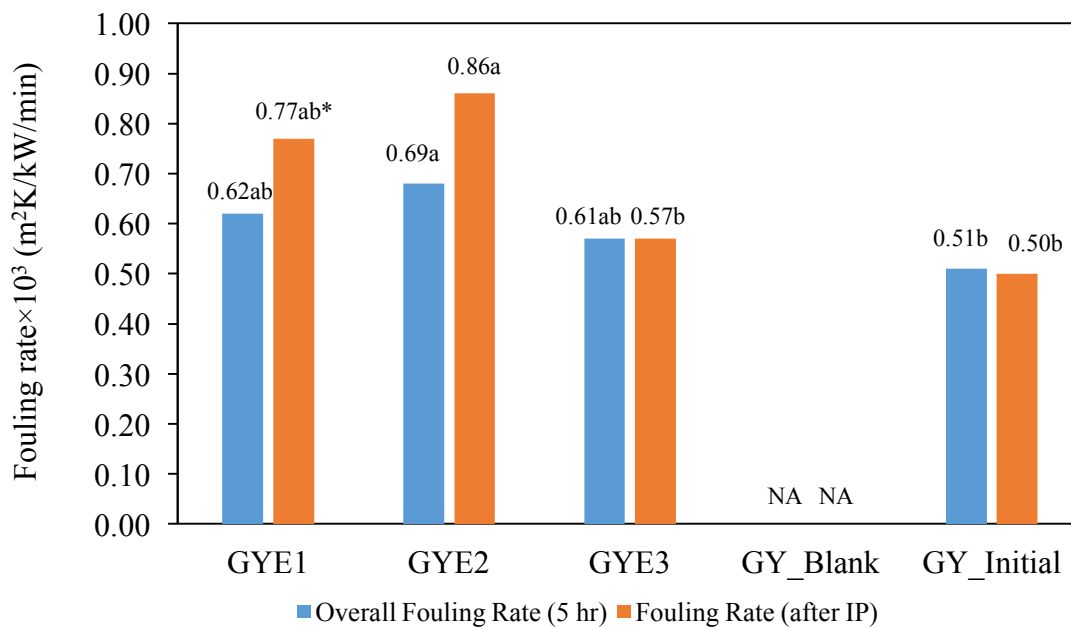
Figure 5.6. Mean fouling rates of glucose-yeast thin stillage fluids in 5 hr and after induction periods.
GYE: glucose-yeast fluids with protease addition (1, 2, 3 g); GY_Blank: glucose-yeast fluids with incubation procedures but without protease; GY_Initial: initial glucose-yeast fluids with no protease and incubation. Asterisk indicates difference in overall fouling rate and fouling rate after IP within each type of treatment; values with same letter in the same color columns are similar, $p < 0.05$.

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Bromelain treated samples displayed fouling tendencies while blank samples resulted in an almost flat fouling resistance curve. GY_Blank (without addition of protease) had lowest R_{max} during 5 hr tests with a mean value lower than those of the other four fluids. Induction periods of blank samples were longer than 300 min since all fouling resistance values were less than 0.05 m^2K/kW. Fouling rates were smaller than 0.1×10^{-3} $m^2K/kW/min$. In contrast to blank samples, incubation with protease increased fouling resistance for all protease treated samples (Fig. 5.5). A small amount of protease (1 and 2 g) was found to extend induction time while more protease addition had similar fouling properties as those of initial samples (Table 5.2).

Addition of small amounts of bromelain failed to reduce R_{max}, but incubation procedures inhibited occurrence of fouling, reflected by the flat fouling resistance curve of blank fluids. Pretreatments between initial and blank fluids led to reduction of fouling properties. The slow growth in fouling can be caused either by low temperature heating or pH adjustment. In comparison of protease treated samples with blank samples, protease addition during incubation facilitated deposit formation with higher R_{max} and fouling rates. It was conjectured that protease itself or hydrolyzed substances, such as peptides and amino acids, increased fouling. Protease effects on heat exchanger fouling have been reported in dairy industry. Enzymes excreted by psychrotrophic bacteria in milk broke down casein micelle, which promoted protein coagulation and deposition formation (Bansal and Chen 2006; Jeurnink 1991). GYE1 and GYE2 had lower fouling resistance values during the first 150 min compared with initial samples, which implied bromelain promotion on fouling was outweighed by effect of incubation pretreatment (Fig. 5.5).

To determine which factors may be responsible for decreased fouling, model fluids made of yeast slurries with or without pH adjustment and preheating were evaluated. Heat incubation time failed to affect fouling properties (Fig. 5.7 and Table 5.3). Thin stillage fluids, with or without heat incubation, displayed fouling phenomena. Fluids with pH adjusted yeast slurries all displayed nearly flat fouling resistance curves (Fig. 5.8). All pH adjusted samples had fouling resistance less than 0.05 m^2K/kW and induction periods longer than 5 hr. Fouling rates of fluids without pH adjustment were more than four times higher than pH adjusted fluids. Fouling was suppressed when pH of fluids (7 L) decreased from 6.2 to 5.5 (Fig. 5.2, Fig. 5.8).
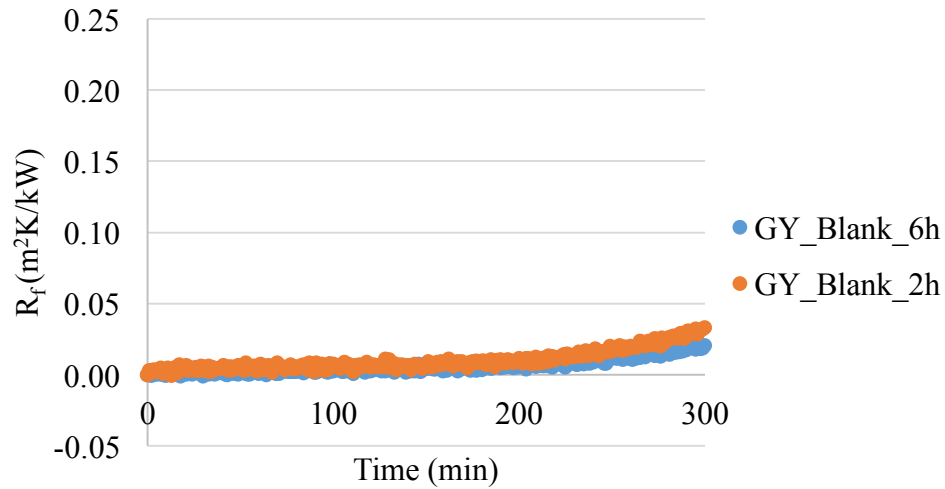
Figure 5.7. Mean fouling resistance curves of glucose-yeast fluids with 2 and 6 hr heat incubation (each line was obtained from two replicates).

Table 5.3. Mean of R_{max} of glucose-yeast fluids with 2 and 6 hr heat incubation.

Treatment	R_{max} (5 hr) (m^2K/kW)
GY_Blank_6h	0.0236±0.0233a
GY_Blank_2h	0.0335±0.0186a

Values with the same letter are similar (P<0.05)
Fouling rates are close to 0 and induction periods are longer than 5 hr (not shown).

Wilkins et al (2006b) investigated pH effect of thin stillage on dry grind heat exchanger fouling and found induction periods decreased when pH decreased from 4.5 to 3.5 but fouling rate increased from pH 4.0 to 4.5. In contrast to Wilkins et al (2006b), glucose-yeast fluids had a higher pH range and larger reduction in fouling rate. When pH of yeast slurries varied from 5.60 to 5.00 (thin stillage fluids pH from 6.30 to 5.50), fouling was negligible with fouling rates less than 0.0001 $m^2K/kW/min$ and induction periods longer than 5 hr. The pH point where fouling reduced also was higher than that of commercial thin stillage, which was reported from pH 3.7 to 4.7 (Wilkins et al 2006b).
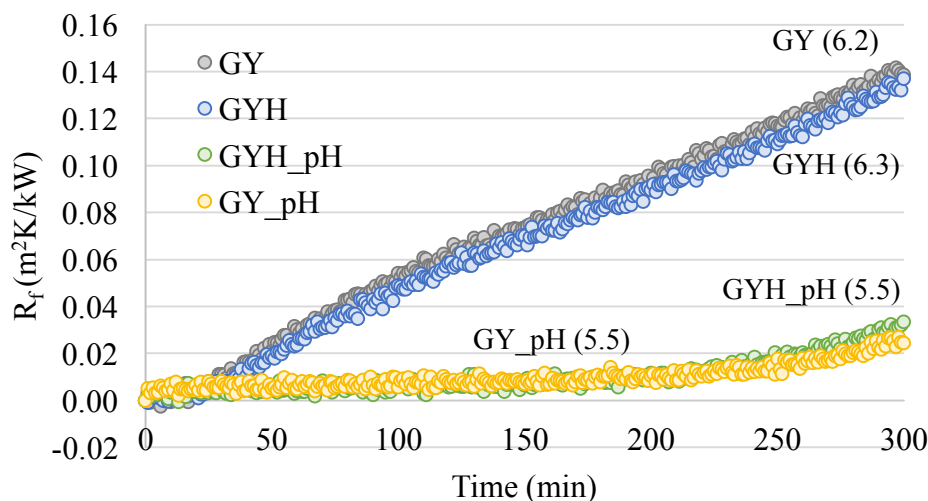
Figure 5.8. Mean fouling resistance curves of glucose-yeast thin stillage fluids with pH adjustment and 2 hr heat incubation.
GY: glucose-yeast fluids without any treatment; GYH: glucose-yeast fluids with yeast slurries that had 2 hr preheat but no pH adjustment; GYH_pH: glucose-yeast fluids with yeast slurries that had 2 hr preheat and pH adjustment; GY_pH: glucose-yeast fluids with yeast slurries that had pH adjustment; means of two replicates.

Due to a decrease in fouling properties of fluids with pH adjustment, it was thought that pH variation inhibited fouling while protease addition expedited fouling. The pH effect on fouling might be related to isoelectric points (pI) of proteins and peptides, which was hypothesized in previous dry grind study by Wilkins (2006b). It was speculated rapid fouling at pH 3.5 was caused by glucoamylase and water-soluble corn protein since the enzyme had pI near 3.5 and corn protein had pI lower than 4.8 (Wilkins et al 2006b). When pH is close to the pI, proteins or peptides with zero net charge are more prone to associate with other protein molecules or even minerals and lipids and promote formation of aggregates. In addition, solubility of proteins and peptides can be affected by number of hydrophobic side groups, length of chains, aqueous conditions like pH, temperature and ion distribution (Wall and Paulis 1978). The suspended particles and instable compounds with conformational changes may cause particulate and chemical reaction fouling (Awad 2011).

The average crude protein of yeast powder was 47.3% while protein content of pellets from incubation samples but without enzymes was lower at 41.1% (Table 5.4). The reduction could be due to dissolution of water soluble protein or other nitrogen compounds, release of ammonia or

nitrogen gas during low temperature incubation though the flasks were sealed by alumina foils. With enzymatic incubation, protein contents of yeast pellets were reduced 15 to 18% in comparison with those of blank samples; therefore, protease hydrolyzed certain amount of proteins into water soluble peptides and amino acids. However, remaining protein concentrations in pellets were similar no matter what amount of protease was added; therefore 1 g protease was enough to break down peptide bonds and change the solubility of some proteins. The similar R_{max} and overall fouling rates among enzyme added samples were in accordance with constant protein contents of pellets. Causes to differences in induction periods and shape of fouling resistance curves among protease added samples were not clear; perhaps it was related to molecule size of hydrolyzed products and number of amino acid unit within peptides.

Table 5.4. Crude protein contents of yeast powder and deposits after protease incubation.

Sample	Treatment	[+] Crude Protein %
Yeast Powder	NA	47.29±0.54a
Pellets_Blank	GY_Blank	41.14±0.30b
Pellets_E1	GYE1	34.76±1.60c
Pellets_E2	GYE2	33.65±3.13c
Pellets_E3	GYE3	33.66±0.87c

Yeast Powder: commercial inactive yeast
Pellets_Blank: yeast slurry deposits obtained by centrifugation after incubation without protease
Pellets_EX: yeast slurry deposits obtained by centrifugation after incubation with Xg protease
[+] Means of three replicates (except for Pellets_Blank with two replicates)
Values with same letter in each column are similar, $p<0.05$.
N% was measured, Protein = N%×6.25.

Fouling properties of model fluids were compared with those of commercial thin stillage. Fouling tendency of GY_Initial was similar to that of TS based on similar R_{max} and fouling rate (Table 5.5). Due to smaller fouling resistance in commercial samples, the induction period of one sample batch was longer than 300 min according to its definition in methods. Therefore, induction period of commercial thin stillage was not used for statistical analysis.

Table 5.5. Comparison of fouling properties among glucose-yeast thin stillage fluids with protease and commercial thin stillage.

Treatment	R_{max} (5 hr) (m^{2}K/kW)	Induction Period (min)	Fouling Rate×10^3 (5 hr) (m^{2}K/kW/min)
GYE1	0.165±0.002a	154.7±10.8a	0.623±0.015a
GYE2	0.199±0.023a	119.0±9.5b	0.687±0.050a
GYE3	0.188±0.008a	76.0±2.6c	0.606±0.048a
GY_Blank	0.0236±0.0233b	>300d	<0.1d
GY_Initial	0.156±0.025ac	95.0±7.8c	0.510±0.094ac
TS	0.0877±0.0486bc	NA	0.284±0.183bc

[+] Means of three replicates for GY, two replicates for TS, values with same letter in each column are similar, p<0.05.
GYE: glucose-yeast fluids with protease addition (1, 2, 3 g)
GY_Blank: glucose-yeast fluids with incubation procedures but without protease
GY_Initial: initial glucose-yeast fluids with no protease and incubation
TS: thin stillage

5.4. Conclusions

Glucose-yeast model fluids with protease incubation showed small variations in fouling properties. Induction periods varied with the amount of protease added for incubation while R_{max} and overall fouling rate were similar. Model blank samples displayed a reduction in R_{max} and overall fouling rate, which was explained by pH adjustment during enzymatic incubation. Preheating had no impact on fouling properties while lowering pH value of glucose-yeast fluids prevented deposit accumulations on heat exchanger surfaces. Reoccurrence of fouling phenomenon with addition of protease was indicative that enzyme itself or hydrolyzed products may be able to promote fouling and accelerate formation of deposits.

CHAPTER 6. PROTEASE AND TOTAL SUSPENDED SOLIDS EFFECT ON HEAT TRANSFER FOULING OF COMMERCIAL THIN STILLAGE

6.1. Introduction

From studies on simplified glucose-yeast model thin stillage fluids in Chapter 5, we have indications of protein effects on heat transfer fouling. Due to much more complicated composition of commercial thin stillage than that of model fluids, studies merely on established models are not enough for the comprehensive understanding of commercial thin stillage fouling properties. Apart from differences in components, other factors such as plant cleaning schedule, evaporator effect and oil skimming might affect fouling tendencies and further alter heat transfer efficiency. For instance, Challa et al (2017) found fouling rate decreased and induction period increased shortly after plant shut down and cleaning; Zhang et al (2017) found that heat treatment of evaporator did not affect fouling properties. Considering influence of fluid composition itself, manufacturing methods and equipment specifications, it is useful to return to commercial thin stillage to understand more comprehensively causes of fouling.

The protein in commercial thin stillage mainly consists of yeast and corn protein; the latter has a higher contribution to total amino acids in downstream coproducts (Han and Liu 2010). Generally, corn protein is divided into four groups, albumin, globulin, prolamin and glutelin. Among them, zein and glutelin are two primary proteins and account for 40 and 37% of corn nitrogen, respectively (Wall and Paulis 1978). Structure and conformation of proteins can be altered by aqueous solution such as addition of acid, base or detergent solution, change in ion concentration, pH and temperature, which either promotes or breaks down covalent or noncovalent bonds and leads to variation in solubility (Wall and Paulis 1978). For example, 70% ethanol soluble zein could be precipitated with higher ion concentration; reducing and alkyl agents are found to cleave disulfide bonds and cause unfolding of protein; phytic acid can form insoluble complexes with albumin under acidic conditions (Craine and Fahrenholtz 1958). To take fluid conditions and potential interactions among diverse compounds into consideration, investigation of protein and protease effect on thin stillage fouling should target commercial samples. In addition, by comparing fouling response of glucose-yeast fluids with that of commercial fluids under the same pretreatment, we are able to evaluate the representativeness of model fluids.

Apart from components' effects on heat transfer fouling, total solids (TS) content in commercial thin stillage also is related with fouling properties. Challa et al (2017) adjusted TS of commercial thin stillage from 7 to 11% obtained from four stages of first effect evaporator and observed higher fouling rate with increased TS. Arora (2010) found fouling resistance reduced by half when solids content decreased from 7.2 to 3.5% without change in composition during 10 hr test. However, from previous glucose-yeast model fluids, addition of glucose to yeast slurries reduced fouling rate by half even though TS were 7% compared with 3.4% of pure yeast fluids. In view of the solubility of glucose, another solid related parameter, total suspended solids (TSS), attracted our interest. In fact, TS include all matters that are suspended and dissolved in fluids while TSS refer to those retained by filters with specific pore size, excluding dissolved substances in permeates (Cleceri et al 1998). Arora (2010) used a microfiltration membrane (0.1 micron pore size) to filter thin stillage and found that microfiltered samples possessed less fouling phenomenon in contrast with diluted fluids that had the same total solids. People from industry also found higher amount of TSS aggravated fouling but without any pertinent research reported. Fouling can be enhanced either by sedimentation or attachment of suspended solids onto heat exchanger surfaces with the aid of gravitation, electrostatic attraction or covalent bonds. Those suspended solids accumulate on the surfaces with longer processing time and form particulate fouling (Arora et al 2010; Bansal and Chen 2006; Visser and Jeurnink 1997).

We studied protein effect on heat exchanger fouling of commercial thin stillage by adding protease bromelain. Considering potential aggregation and coagulation of different particles, variations in TSS and fouling properties with storage time were tracked and relationships between TSS and fouling characteristics were explored.

6.2. Materials and Methods

6.2.1 Commercial thin stillage with protease incubation

Two batches of commercial thin stillage were collected from a dry grind plant and stored at room temperature (20±5°C) before fouling tests. To avoid potential impact from aging, fouling tests were run within 14 days after collection. Each batch of samples had three treatments (TSi, TSb and TSe), the volume of each was 7 L as in previous studies (Fig. 6.1, Table 6.1). TSi (thin stillage initial) was thin stillage directly used for fouling test. TSb (thin stillage blank) was

sample that went through incubation procedures without enzyme addition. After incubation, thin stillage fluid temperature was increased from 48 to 80°C. Protease was deactivated during heating over 70°C. TSe (thin stillage enzyme) was thin stillage with addition of 3 g bromelain (Sigma Aldrich, >= 3.0 units/mg, >= 35% biuret) for 6 hr incubation at 48°C under natural pH before fouling test. Protease was deactivated using the same method as TSb.
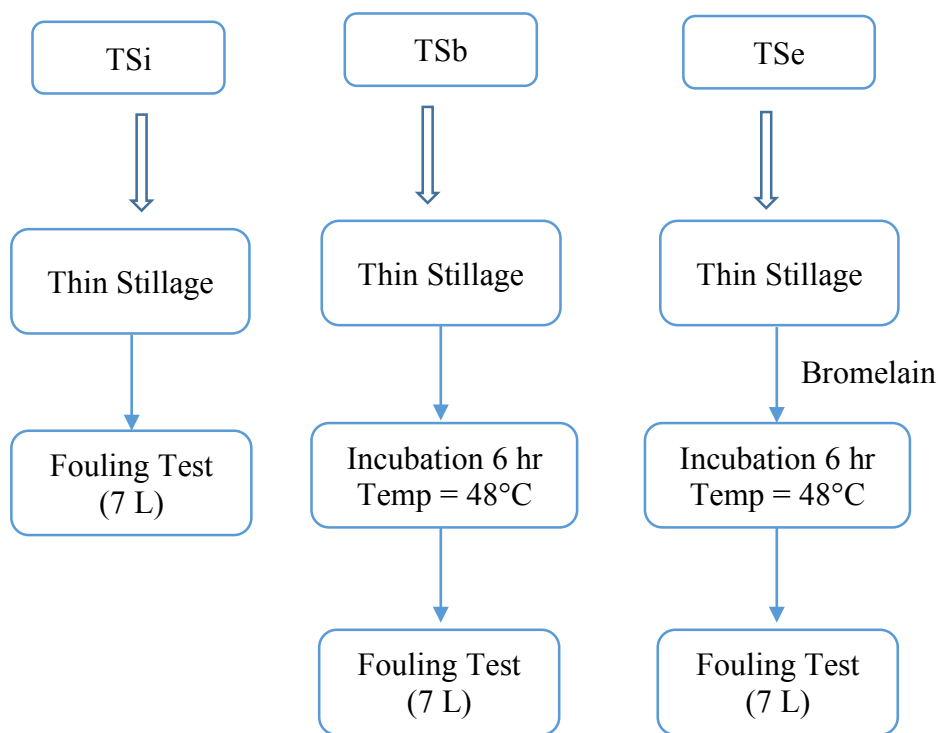
Figure 6.1. Processing flowchart of three treatments.

Table 6.1. Two batches of commercial samples from dry grind plant.

Treatment	Protease (g)	+Total Solids (%)	+pH	Incubation Time (hr)	Collection date
TSi1	NA	7.61	5.25	0	2017/02/15
TSb1	NA	7.61	5.20	6	2017/02/15
TSe1	3	7.61	5.18	6	2017/02/15
TSi2	NA	7.79	5.22	0	2017/03/02
TSb2	NA	7.79	5.22	6	2017/03/02
TSe2	3	7.79	5.21	6	2017/03/02

Each batch of commercial samples had 7 runs.
TSi: original commercial thin stillage for fouling tests (three replicates)
TSb: thin stillage with 6 hr incubation but without addition of protease (two replicates)
TSe: thin stillage with 6 hr emzymatic incubation (two replicates)
+ Means of pH values and total solids

6.2.2. Fouling Test

Before incubation and fouling test, total solids (TS) and pH of commercial fluids were characterized. TS measurement was conducted by a standard method (AACCI 2000) and pH was detected by a portable pH meter (Waterproof pH 150, Oakton, Vernon Hills, IL). For each batch of samples, TS were 7.61 and 7.79% (db) and pH was in the range of 5.05 to 5.25, which was close to the pH used for enzymatic incubation in Chapter 5 (Table 6.1). After incubation, fouling tests were conducted. The same annular fouling apparatus, cleaning protocols and operating conditions were used as described in Chapter 3. Viscosity was measured at 75°C and fluid velocity was recorded during tests. Re was found to be 830±10 (Appendix C). Fouling was characterized by the same parameters in Chapter 3 except that induction period was defined as the time during which averages of three continuous fouling resistance were less than $0.01 m^2 K/kW$. To avoid potential aging effects, three replicates for each treatment with shorter storage times (less than 10 days) were selected from all commercial samples to explore protease impact on fouling. Analysis of variance (ANOVA) and Tukey's honest significance test (R version 3.2.2) were used for fouling parameters. The statistical significant level was 5% ($p < 0.05$).

6.2.3 Total suspended solids (TSS) measurement

For each batch of samples, three subsamples (7 L) were run for fouling tests on the first, third and seventh days after collection. Using two replicates, TSS (EPA Method 160.2) were measured on different storage days. Whatman 934-AH Glass Microfiber filters (particle retention 1.5 μm, Fisher Scientific) were used for separation with 50 ml loading sample. Due to practical range of TSS measurement, subsamples were diluted 50× before filtration. Fouling properties as well as relationships between TSS and storage times were studied.

6.3. Results and Discussion

For the first batch, fouling resistance increased with the experiment time. TSi1 were three initial thin stillage samples with the least fouling resistance (Fig. 6.2). Fouling resistance curves of TSe1 fell between two TSb1 samples with fouling rate at 1.4×10^{-3} $m^2 K/kW/min$. Induction periods of TSb1 and TSe1 were shorter than TSi1, decreased by 90 and 84%, indicating more rapid fouling occurred of incubated samples. Compared with TSi1 and TSe1, two fouling

resistance curves of TSb1 displayed a deviation. The CV of R_{max}, induction period and fouling rate were larger than 80% for TSb1.
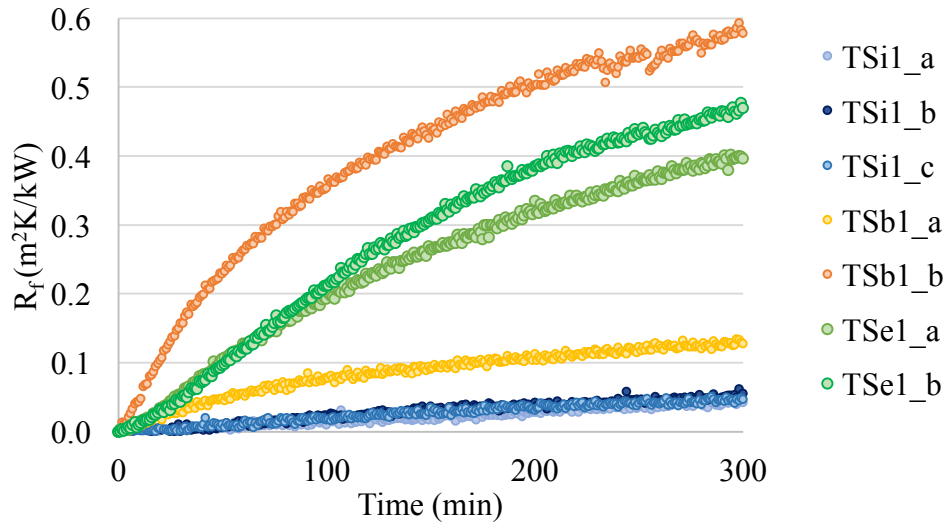
Figure 6.2. Commercial thin stillage fouling resistance with different treatments (first batch). TS: thin stillage; TSi: without incubation and addition of protease; TSb: with incubation procedure without protease; TSe: with enzymatic incubation; lower case letters a, b, c represent replicates.

For the second batch of samples, TSi2 had the least steep fouling resistance curves with average fouling rate was 0.4×10^{-3} m^2K/kW/min. Additional incubation before fouling test increased the R_{max} as well as fouling rate, which indicated deposits were formed at a faster rate. In comparison with TSb2, addition of protease further accelerated fouling, shown by two steepest green curves (Fig. 6.3). R_{max} and fouling rate of TSe2 were higher than TSi2 and TSb2. From 2nd batch, it was found that protease promoted occurrence of fouling and this phenomenon also was noted in glucose-yeast model fluids.
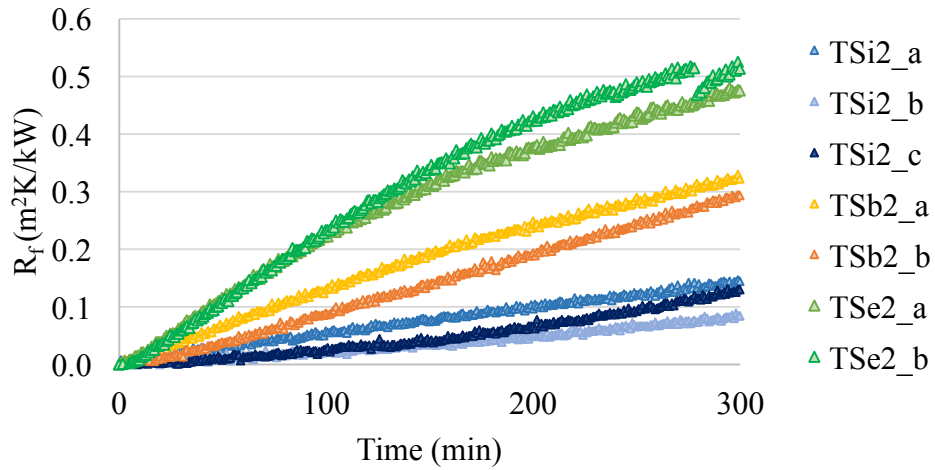
Figure 6.3. Commercial thin stillage fouling resistance with different treatments (second batch). TS: thin stillage; TSi: without incubation and addition of protease; TSb: with incubation procedure without protease; TSe: with enzymatic incubation; lower case letters a, b, c represent replicates.

From mean fouling resistance curves of two commercial fluids, incubated samples (with or without protease) displayed higher fouling rates and R_{max} than initial ones during 5 hr (Fig. 6.4). Fouling resistance curves of TSb1 and TSi1 intersected during 100 and 150 min, indicating that deposits produced more quickly at the beginning in blank fluids. This phenomenon was affected primarily by one extreme data in TSb1, which increased average R_{max} and fouling rate.
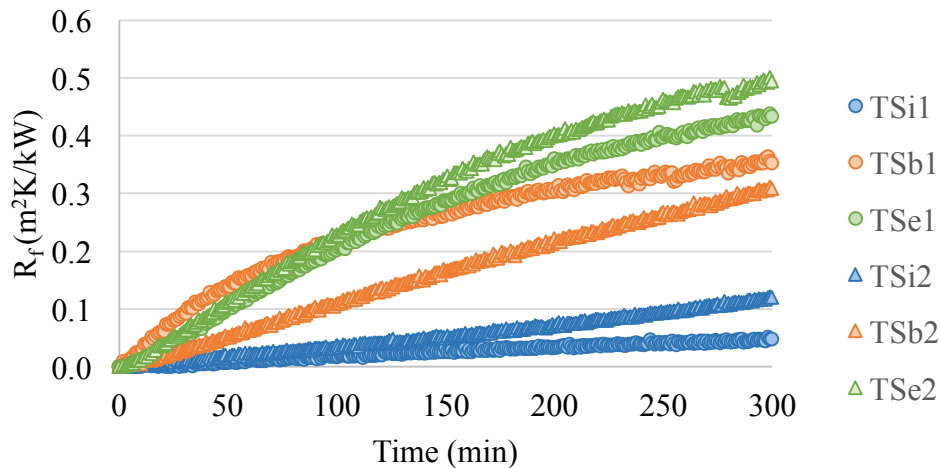

Figure 6.4. Average fouling resistance curves of two batches of commercial thin stillage with different treatments.
TS: thin stillage; TSi: without incubation and addition of protease; TSb: with incubation procedure without protease; TSe: with enzymatic incubation.

Three replicates with shorter storage times for each treatment were combined to investigate effects of protease on fouling properties. Addition of protease increased R_{max} and fouling rate by four times in comparison with initial commercial fluids (Table 6.2). By comparing incubated fluids (no protease) with initial fluids, it was found that incubation procedures affected fouling, which was reflected by increased fouling rate in commercial samples and infinite small R_{max} and fouling rate in glucose-yeast model fluids. The accelerated fouling tendency in TSb might be caused by low temperature preheating or long time circulation within the system, where testing fluids flowed over the probe surface repeatedly and accumulated compounds promoting later deposition. The flat fouling curves of model fluids were attributed to pH adjustment defined in Chapter 5. Therefore, comparisons between enzymatic incubated and blank samples better explained protease impact on fouling excluding other influential factors. Model fluids with enzymatic incubation presented higher fouling trends than blank samples, which was reflected by three fouling parameters. The increase in fouling between TSb and TSe was less obvious than that of model fluids with a difference in R_{max} (Table 6.2, Fig. 6.4). R_{max} and fouling rate of TSe were more than twice as GYe with the same amount of protease (3 g). Different components between glucose-yeast mixtures and thin stillage may have various chemical reactions or structure and conformation changes when heated, as a result of different fouling properties.

Table 6.2. Fouling properties of commercial thin stillage and glucose-yeast model fluids[+].

Treatment	R_{max} (5 hr) (m^2K/kW)	Induction Period (min)	Fouling Rate$\times 10^3$ (5 hr) $(m^2K/kW/min)$
TSi	0.0855±0.0537a	42.3±26.7a	0.257±0.180a
TSb	0.253±0.104a	12.3±4.9a	0.805±0.391ab
TSe	0.469±0.062b	7.7±1.5a	1.49±0.21b
GYi	0.156±0.025A	95.0±7.8A	0.510±0.094A
GYb	0.0236±0.0233B	>300.0B	<0.1B
GYe	0.188±0.008A	76.0±2.6C	0.606±0.048A

[+] Mean of three replicates from each treatment
Values with the same letter in each column are similar ($p<0.05$).
TS: thin stillage; GY: glucose-yeast model fluids
TSi/GYi: without incubation and addition of protease
TSb/GYb: with incubation procedure without protease
TSe/GYe: with enzymatic incubation

For each batch of commercial thin stillage, four subsamples were taken on the 1st, 3rd, 7th and 14th day after collection for total suspended solids (TSS) measurement, fouling tests were conducted within one week. There was a slight decrease in pH with a longer storage time, varying from 5.29 to 5.18 for 1st batch and 5.25 to 5.19 for 2nd batch from day 1 to 7 (Table 6.3). TSS during two weeks were similar by one-way ANOVA ($p<0.05$) for each batch, which indicated TSS kept constant within two week period (Fig. 6.5). The same trends also were noted in R_{max}, fouling rate and induction period, which implied one week storage time failed to affect both TSS and fouling parameters. A similar observation of thin stillage aging was reported from Zheng's research (2013), where fouling properties did not change over 20 days. However, the sample volume utilized (30 L) was much higher than that in this study (7 L) with bulk temperature and probe temperature at 48 and 100°C, respectively.

Table 6.3. Fouling properties of commercial thin stillage within one week.

Treatment	pH	R_{max} (5 hr) (m^2K/kW)	Induction Period (min)	Fouling Rate$\times 10^3$ (5 hr) (m^2K/kW/min)
TS1_1	5.29	0.048	59.0	0.140
TS1_3	5.28	0.062	54.0	0.168
TS1_7	5.18	0.050	59.0	0.157
TS2_1	5.25	0.147	14.0	0.464
TS2_3	5.23	0.089	40.0	0.312
TS2_7	5.19	0.131	44.0	0.464

TSx_y: thin stillage; x is batch number; y is storage time before fouling test.
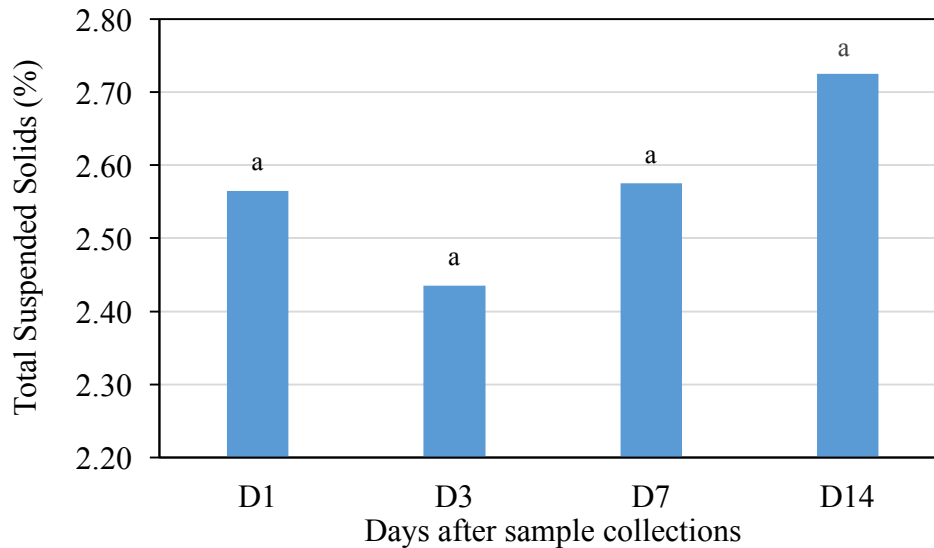
Figure 6.5. Total suspended solids (TSS) of two batches of commercial thin stillage with different different storage time.
The same letter indicates TSS are similar with storage time (p<0.05).

Seven day storage had little impact on fouling properties and TSS but there were variations between these two batches (Table 6.4). Average TSS of TS2 was higher than that of TS1 with 2.78 and 2.27%, respectively. Considering differences in total solids of these two batches, the relative TSS was calculated, which was the ratio of total suspended solids to total solids. They were 0.298 and 0.357, a higher TSS proportion in TS2. The average fouling rate and R_{max} also varied between these two batches (Fig. 6.6). R_{max} and fouling rate of TS2 were twice those of TS1 indicating the potential relationship between TSS and fouling properties (Table 6.5). Arora (2010) investigated microfiltration's effect on thin stillage fouling tendencies and obtained 3.5% total solids in permeate, which were counted as dissolved solids. The average relative TSS for his research was 0.462, higher than our samples. It can be due to smaller membrane pore size he used (0.1 μm). There was a fouling reduction in thin stillage permeate with certain amounts of protein and fat retained on the filter, which strengthened former speculation that TSS makes a contribution to fouling.
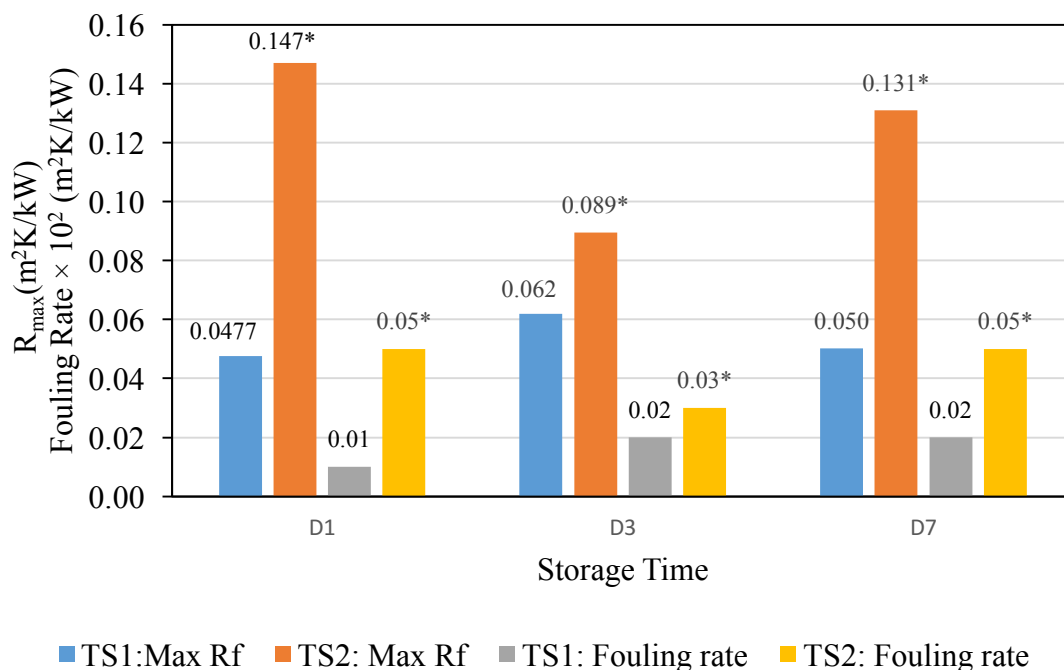
Figure 6.6. Max R_f and fouling rate of two batches thin stillage samples on different storage days. Asterisk indicated there are differences in R_{max} and fouling rate from batch to batch.

Table 6.4 Fouling variations of two batches commercial thin stillage in one week[+].

Storage time	R_{max} (5 hr) (m^2K/kW)	Induction Period (min)	Fouling Rate$\times 10^3$ (5 hr) ($m^2K/kW/min$)
Day 1	0.0973±0.0703a	36.5±31.8a	0.302±0.229a
Day 3	0.0757±0.0194a	47.0±9.9a	0.240±0.102a
Day 7	0.0905±0.0570a	51.5±10.6a	0.312±0.217a

[+] Mean of two replicates
Values with the same letter in each column are similar ($p<0.05$)

Table 6.5. Total suspended solids and fouling properties of two batches commercial thin stillage in one week[+].

Treatment	TSS	R_{max} (5 hr) (m^2K/kW)	Induction Period (min)	Fouling Rate$\times 10^3$ (5 hr) ($m^2K/kW/min$)
TS1	2.27±0.11a	0.0533±0.0076a	57.3±2.9a	0.155±0.014a
TS2	2.78±0.07b	0.122±0.030b	32.7±16.3a	0.413±0.088b

[+] Mean of three replicates
Values with the same letter in each column are similar ($p<0.05$)

To evaluate interactions between these parameters: R_{max}, fouling rate, induction period, TSS, pH and TS, correlation calculations were conducted in R (version 3.2.2) using Pearson method. The highest correlation factor was between R_{max} and fouling rate and a negative correlation was found between R_{max} (or fouling rate) and induction period. There was a linear relationship between TSS and TS with correlation factor at 0.96. Among TSS, pH, TS, both TSS and TS had linear correlations with fouling properties (Table 6.6).

Table 6.6. Correlations look among fouling properties, TSS, pH and total solids by Pearson method in R.

	R_{max}	Fouling rate	Induction period	TSS	pH	TS
R_{max}	1.00	0.98	-0.88	0.89	-0.34	0.89
Fouling rate	0.98	1.00	-0.80	0.89	-0.52	0.87
Induction period	-0.88	-0.80	1.00	-0.81	0.01	-0.79
TSS	0.89	0.89	-0.81	1.00	-0.49	0.96
pH	-0.34	-0.52	0.01	-0.49	1.00	-0.39
TS	0.89	0.87	-0.79	0.96	-0.39	1.00

Variations of fouling properties were affected more by sample batch than storage time within one week. The possible influential factors can be TS, TSS, sampling time, diverse compositions led by differences in source materials, added components in processing and equipment cleaning schedule. For instance, Challa et al (2017) reported reduction in fouling behaviors after plant shutdown for evaporator cleaning. They found induction periods longer than 5 hr and disappearance of sloughing with samples collected one week after cleaning. Therefore, to avoid variations existing in original thin stillage samples, it is recommended to collect samples within an intensive period of time and avoid overall plant cleaning. If a plant has a routine cleaning for evaporators every one or two weeks, a fixed sampling time with the same amount of days after cleaning may prevent some variations. Adjusting TS, TSS, pH to the same level before fouling tests and meanwhile keeping Re constant during tests also can reduce variations from batch to batch. Moreover, model thin stillage fluids have their advantages for fouling studies owing to their constant and stable composition, easy accessibility and freshness. The disadvantages of model fluids can be ignorance of impacts from substances with tiny quantities and omission of combined or synergistic effects from thin stillage mixtures and fluids' conditions.

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6.4. Conclusions

Bromelain incubation of commercial thin stillage increased fouling properties in comparison with initial and heat incubated samples. The severity of fouling phenomenon was TSe > TSb, which also was observed in glucose-yeast model fluids. Hydrolyzed products peptides, free amino acids or protease speed up the occurrence of fouling rather than mitigate this phenomenon. With that observation, we need to pay more attentions to process retrofits before evaporation, especially applications of enzymes to increase yields and quality of coproducts, and evaluate modification impacts on fouling properties. Apart from TS, TSS can be another important factor related with fouling properties. TSS did not vary during two week storage time at room temperature. For two batches of commercial samples, fouling properties and TSS were more affected by sample batch.

CHAPTER 7. RECOMMENDATIONS

1. To study protein effect on fouling, besides glucose-yeast model fluids, corn steeping water, wet cake or gluten meal soaking water with extracted water soluble protein can be used for fouling tests. Models should be used in a short period of time to avoid aging, microorganism contaminations and potential bioactivities.

2. Investigation of protease impact on heat transfer fouling should extend to a broad candidate of enzymes to: 1) figure out some proteases that might reduce fouling tendencies, 2) study protease hydrolysis effect on fouling and evaluate the value of enzymatic techniques from an overall view and 3) analyze composition and structure variations before and after protease incubation to determine primary fouling contributor.

3. For commercial thin stillage samples, try to avoid variations from batch to batch by collecting samples at a fixed time, storing samples at the same place under similar temperature, adjusting total solids or total suspended solids, pH to the same level and keeping Reynolds number constant during fouling tests.

4. Effect of pH on commercial thin stillage can be studied in a wider range rather than just several points. Extreme pH conditions may bring some unexpected responses favorable for fouling reduction; more interval points will help predict fouling tendencies accompanied by pH variation more precisely.

5. Compositional analysis of thin stillage fluids before and after tests may assist us to locate component most related with fouling and predict substances left in deposits. Deposit analysis, including chemical and imaging techniques, also helps us to understand and explain fouling procedures. Plate heat exchanger or stainless steel coupons make it feasible to visualize various layers of deposits in a microscale to trace deposit formation.

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APPENDIX

APPENDIX A. FOULING TEST PROCEDURES

1. Clean the annular fouling probe to remove visible deposits by sponge dipping with 3% (w/v) NaOH solution and rinse the probe with 2 L tap water.
2. Check the annular fouling apparatus for leaks by circuiting hot water within the system.
3. Clean the fouling system by circulation of 10 L 1% (w/v) Alconox solution for 20 min, 10 L hot tap water for 15 min and hot water rinse for 15 min (keep water input and output rates constant). System is rinsed by hot tap water three times between three steps.
4. Turn on water bath to heat water for heat exchanger.
5. Charge the tank with known volume (7 L) of test fluid.
6. Turn on the pump to circuit test fluid from tank, through heat exchanger, fouling probe and back to tank.
7. Connect the thermocouple extensions (male) from the datalogger to the female ends of fouling probe.
8. Wait until heat exchanger fluid and test fluid reach equilibrium and specific temperature.
9. Collect 30 ml subsample for analysis.
10. Turn on the data logger and collect data for the test fluid.
11. Turn on the power to 220V power supply to fouling apparatus and adjust average probe surface temperature to 120°C by turning the knob connected to limit controller.
12. End experiment by stopping data collection, switching off datalogger, disconnecting 220V power to the probe, turning off water bath and recircuiting pump for test fluid.
13. Discard test fluid to drain, scrub inner walls of tanks with brush and rinse fouling system with hot tap water three times.
14. Remove the thermocouple connections and dismantle the annular probe.
15. Soak the probe in 3% (w/v) NaOH solution overnight.

APPENDIX B. TOTAL SUSPENDED SOLIDS MEASUREMENT

Based on EPA Method # 160.2

A. Fiber preparation

1. Place glass fiber filter on filter apparatus with wrinkled surface up. With applied vacuum, wash the disc with three successive 20 ml volumes of distilled water.

2. Remove all the water by applying vacuum and take glass filter off the filter apparatus.

3. Dry the glass filter in an oven at 103 to 105°C for an hour and put it in desiccator to store until needed.

4. Repeat drying cycle until a constant weight is obtained (weight loss less than 0.5 mg).

5. Weigh immediately before use.

B. Loading & filtering samples

1. Assemble filtering apparatus and begin suction.

2. Place filter onto the filter apparatus and wet it with small volume of distilled water to seat it against the fritted support.

3. Shake the thin stillage sample vigorously and dilute it by 50 times with distilled water. 50 ml diluted solution is loaded to filter using a graduated cylinder. Continuing to apply vacuum to remove all water.

4. With suction on, wash the graduate cylinder, filter and nonfilterable residue and filter funnel wall with three portions (60 ml in total) of distilled water allowing complete drainage before washing. Remove all water by continuing to apply vacuum.
(Total volume of wash water should equal to 2 ml/cm^2.)

C. Drying & weighing residues

1. Carefully remove the filter and dry at least one hour in a hot oven at 103 to 105°C.

2. Cool in a desiccator and weigh.

3. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

D. Calculations:

Nonfilterable residue, $\text{mg/L} = \frac{(A-B)\times 1000}{C}$

Where A = weight of filter + residue in mg

B = weight of filter in mg

C = ml of sample filtered

APPENDIX C. VISCOSITY AND REYNOLDS NUMBER

Table C.1. Glucose-urea model fluids[+].

Treatment	TS (%)	Viscosity (cP)	Re
GL	7	7.50	934
GU23	7	7.75	874
GU50	7	7.50	813
ST	1	8.00	907
STU	1	8.00	861

[+]Mean of viscosity and Re.

Table C.2. Glucose-yeast model fluids[+].

Treatment	TS (%)	Viscosity (cP)	Re
GY17	7	8.50	1207
GY23	7	8.50	1196
GY28	7	8.25	1214
PY23	3.4	8.00	1251

[+]Mean of viscosity and Re.

Table C.3. Glucose-yeast model fluids with protease[+].

Treatment	TS (%)	Viscosity (cP)	Re
GYE1	7	8.17	1226
GYE2	7	8.17	1226
GYE3	7	8.17	1226
GY_Blank	7	8.17	1226
GY_Initial	7	8.00	1251

[+]Mean of viscosity and Re.

Table C.4. Thin stillage with protease[+].

Treatment	pH	Viscosity (cP)	Re
STi	5.26	12.00	832
STb	5.18	12.00	832
STe	5.11	12.13	826

[+]Mean of pH, viscosity and Re. Fluids (7 L) pH after enzymatic incubation.

APPENDIX D. EFFECTS OF COMMERCIAL YEAST POWDERS ON FOULING

Two batches of commercial yeast sample displayed different fouling resistance profiles. The first batch of yeast-glucose model fluids gave linear fouling resistance ($R^2 > 0.99$) with induction period less than 2 hr. Fouling curves of three treatments (marked by triangles) overlapped and fouling rates were between 0.0005 and 0.0006 m^2K/kW/min within the 5 hr test despite different protein concentration levels. There was no replicate for each treatment. The second batch of yeast samples also exhibited increasing fouling resistance in 5 hr test periods. But treatments from the second batch had longer induction periods compared with the first batch (Fig. D.1).
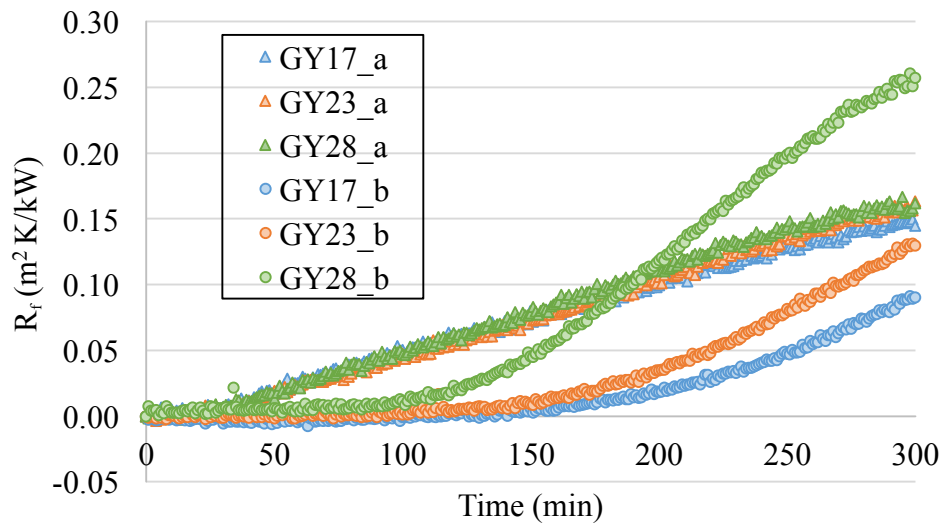

Figure D.1. Fouling resistance of glucose-yeast model fluids at different protein levels. Glucose yeast mixture: 17, 23 and 28% crude protein db; lower case letter a, b represents 1st batch and 2nd batch.