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Incorporation of whey permeate, a dairy effluent, in ethanol fermentation to provide a zero waste solution for the dairy industry

Archana Parashar,^{*1} Yiqiong Jin,^{*1} Beth Mason,[†] Michael Chae,^{*} and David C. Bressler^{*2}

^{*}Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, T6G 2P5, Canada

[†]Verschuren Centre for Sustainability in Energy & the Environment, Cape Breton University, Sydney, B1P 6L2, Nova Scotia, Canada

ABSTRACT

This study proposes a novel alternative for utilization of whey permeate, a by-product stream from the dairy industry, in wheat fermentation for ethanol production using *Saccharomyces cerevisiae*. Whey permeates were hydrolyzed using enzymes to release fermentable sugars. Hydrolyzed whey permeates were integrated into wheat fermentation as a co-substrate or to partially replace process water. Cold starch hydrolysis-based simultaneous saccharification and fermentation was done as per the current industrial protocol for commercial wheat-to-ethanol production. Ethanol production was not affected; ethanol yield efficiency did not change when up to 10% of process water was replaced. Lactic acid bacteria in whey permeate did not negatively affect the co-fermentation or reduce ethanol yield. Whey permeate could be effectively stored for up to 4 wk at 4°C with little change in lactose and lactic acid content. Considering the global abundance and nutrient value of whey permeate, the proposed strategy could improve economics of the dairy and biofuel sectors, and reduce environmental pollution. Furthermore, our research may be applied to fermentation strategies designed to produce value-added products other than ethanol.

Key words: dairy by-product, cheese whey, whey permeate, ethanol fermentation, *Saccharomyces cerevisiae*

INTRODUCTION

Whey is a by-product of cheese and casein manufacturing in the dairy industry, and represents about 85% of the total milk used in the process (Panesar and Kennedy, 2012). Lactose and protein are the major components in whey and account for approximately 75 and 10% of the TS, respectively (Mawson, 1994). Whey

protein is extracted from whey by ultrafiltration. The remaining liquid, whey permeate, is composed mainly of lactose, salts, nonprotein nitrogen, and water (Jelen, 2009). Whey permeate has limited applications, and a major portion of the whey permeate produced in the world is currently being discarded as a dairy effluent. Large amounts of whey are produced annually because approximately 9 kg of whey is obtained per kilogram of cheese produced (Kosikowski, 1979). The world trade of whey and whey products for 2014 was estimated at 5.6 million tonnes, of which the major product was whey powder (Sossna, 2014a). Cheese manufacturing is projected to reach 25.3 million metric tonnes by 2023 (OECD-FAO, 2014), which would result in enormous amounts of available whey permeate. In 2013, the global market for whey powder and proteins was estimated at \$9.8 billion US and was forecasted to be worth \$11.7 billion US by 2017 (Sossna, 2014b). Therefore, finding novel ways to use whey permeate is crucial.

Current practices followed by the dairy industry for dealing with whey permeate include disposing of it as waste, land spreading, selling dry permeate powder, and incorporation into animal feed. The use of whey permeate as a liquid additive in livestock feed has several inherent limitations, and most importantly, this market is slowly disappearing. Although whey permeate is biodegradable, its release into the environment contributes significantly to land and water pollution due to its high biochemical oxygen demand (40,000–48,000 mg/L) and chemical oxygen demand (80,000–95,000 mg/L; Cotanch et al., 2006; Kushwaha et al., 2011). Effective disposal often requires extensive pretreatments and therefore contributes to the operating costs of the dairy plant (Marwaha and Kennedy, 1988). Moreover, handling large volumes of surplus whey permeate is not profitable and is a critical issue that the industry is facing. Dairy plants worldwide are currently looking for alternative strategies for cost-effective use of whey permeate.

The use of whey permeate as a direct lactose source has been implemented in some dairy facilities; however, this requires extensive processing, including demineral-

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¹These authors contributed to this work equally.

²Corresponding author: david.bressler@ualberta.ca

alization and dewatering (Jelen, 2009). One promising avenue for direct use of whey-based lactose is in biofuel production. Several efforts have been made in the past to use whey as a fermentation substrate for ethanol production, including a few industrial-scale plants in New Zealand, United States, and Denmark (Lyons and Cunningham, 1980; USDA Rural Business and Cooperative Programs, 2008; Guimarães et al., 2010). However, low ethanol yields due to low lactose content and high process costs (such as reverse osmosis for concentrating whey) made the use of whey permeate economically unattractive and limited its widespread application. Most processes use *Kluyveromyces* strains to directly convert lactose to ethanol, but this genus of yeast has major drawbacks including low ethanol titers of 2.5 to 4.2% (vol/vol), low osmotic tolerance, and prolonged fermentation times (USDA Rural Business and Cooperative Programs, 2008; Guimarães et al., 2010). Development of novel alternative technologies that require minimal preprocessing would not only improve overall economics for the ethanol industry, but would also create a new value-added market for this surplus dairy waste stream and reduce the environmental burden of whey permeate disposal.

One of the major problems for the ethanol industry is contamination of the fermentation process by lactic acid bacteria, which could be a primary issue associated with using whey permeate for ethanol production. It has been reported that lactic acid bacteria are the natural microorganisms present in whey (Wongso, 1993). These bacteria compete with yeast for carbon sources and produce acids that are toxic to the yeast, both of which result in decreased ethanol yields. These contamination events in industrial fermentations can result in a 27% loss in fermentation efficiency or can lead to a complete fermentation failure at the batch level (Bischoff et al., 2009). The contamination issue is becoming of greater importance as the bioethanol industry moves toward lower energy systems, including the reduction or elimination of jet cooking and lower temperature hydrolysis methods for grain feedstocks. Therefore, it is important to investigate and evaluate the potential negative effect of supplementing whey permeate containing lactic acid bacteria for ethanol production.

The aim of this study was to determine the feasibility of integrating whey permeate into the conventional grain-to-ethanol fermentation process. Specifically, we investigated if whey permeate could be used as an inexpensive co-substrate or partial process water replacement in ethanol fermentations without affecting the ethanol yield. Cold starch hydrolysis-based simultaneous saccharification and fermentation were performed using current industrial protocols for wheat-to-ethanol

production. Whey permeate was procured from a large dairy producer from 2 different cheese manufacturing plants, and multiple batches were tested to validate the developed process. *Saccharomyces cerevisiae*, which is a favorable microorganism for industrial ethanol production, was used for fermentation due to its well-established fermentative capabilities such as high tolerance to substrate and product inhibition (Cot et al., 2007).

MATERIALS AND METHODS

Materials

Chemicals including glucose, galactose, lactic acid, and acetic acid (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Sulfuric acid (HPLC grade) was acquired from Fisher Scientific (Fairlawn, NJ). The enzymes STARGEN 002 (cocktail of *Aspergillus kawachii* α -amylase and *Trichoderma reesei* glucoamylase), Optimash TBG (thermostable β -glucanase), GC 626 (acid α -amylase), and Fermgen (protease) were kindly provided by Genencor International (Palo Alto, CA). Viscozyme Wheat (cocktail of xylanase and pentosanase), Liquozyme SC (α -amylase), and Spizyme SC DS (glucoamylase) were kindly provided by Novozymes (Franklinton, NC). Lactozyme 3000 L, a β -galactosidase from *Kluyveromyces lactis*, was purchased from Sigma-Aldrich (St. Louis, MO). Spring wheat (AC Andrew) was kindly provided by Seed Solutions (Viking, AB). Wheat was ground by dry-milling (grain only) using a laboratory hammer mill (model 3100, Perten Instruments, Sweden) equipped with a mill feeder (model 3170, Perten Instruments, Sweden), and sieved (0.5 mm) before use in fermentations. Whey permeate by-product effluent was procured in multiple batches from a large dairy producer in its liquid form (without any modifications) from 2 different Canadian cheese manufacturing plants. *Saccharomyces cerevisiae*, Superstart active distillers dry yeast, generously provided by Lallemand Ethanol Technology (Milwaukee, WI), was used as the fermenting organism to produce ethanol.

Compositional Analyses

Chemical profiling of the fermentation samples was done as per standard protocols. Lactose, glucose, galactose, lactic acid, and acetic acid content was determined using an Agilent 1200 series HPLC instrument (Agilent, Santa Clara, CA), with a refractive index detector, Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) at 60°C (300 \times 7.8 mm), using 5 mM sulfuric acid as the mobile phase with a flow rate of 0.5 mL/min. Alternatively, for samples with less than 1

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