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Development of an Oat-Based Biorefinery for the Production of L(+)-Lactic Acid by *Rhizopus oryzae* and Various Value-Added Coproducts

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A novel oat-based biorefinery producing L(+)-lactic acid and various value-added coproducts (e.g., β-glucan, anti-irritant solution) is proposed. Pearling is employed for sequential separation of bran-rich fractions for the extraction of value-added coproducts. Lactic acid production is achieved via fungal fermentation of *Rhizopus oryzae* on pearled oat flour. Maximum lactic acid concentration (51.7 g/L) and starch conversion yield (0.68 g/g) were achieved when an oat flour concentration of 116.5 g/L was used. Oxygen transfer played a significant role with respect to lactic acid production and starch conversion yield. *Rhizopus oryzae* produced a range of enzymes (glucoamylase, protease, phosphatase) for the hydrolysis of cereal flour macromolecules. Enzyme production during fungal fermentation has been reported. The proposed biorefining strategy could lead to significant operating cost reduction as compared to current industrial practices for lactic acid production from pure glucose achieved by bacterial fermentations.

KEYWORDS: Oat-based biorefinery; lactic acid production; *Rhizopus oryzae*; fungal fermentation

INTRODUCTION

The industrial implementation of sustainable and viable biomass-based biorefineries depends on the development of integrated processing strategies that exploit the full potential of complex biological entities for the production of a wide spectrum of products (*1*). Some examples of current biorefining schemes utilizing various biomass-derived feedstocks that are already in operation or under development include bioethanol, polylactic acid, 1,3-propanediol, and polyhydroxyalkanoates from corn; bioethanol from straw; and epichlorohydrin from rapeseed oil (*2*).

Lactic acid is used for both food and nonfood applications including cosmetics, pharmaceuticals, and chemical production. Lactic acid is currently produced via bacterial fermentation from corn as a platform chemical for the production of the biodegradable polymer, poly lactic acid (PLA). PLA is used as an environmentally benign substitute for petrochemically derived plastics as well as in some medical applications. Lactic acid can also be produced directly via fungal fermentation carried out mainly by *Rhizopus* species (*3*−*7*).

The development of fungal fermentations for the production of lactic acid will lead to significant cost reduction. Fungal bioconversions can be more economic as compared to bacterial bioconversions because fungi can hydrolyze complex macromolecules (starch, protein, phytic acid) contained in cereals directly into assimilable monomers. The simultaneous hydrolysis of cereal grains and lactic acid production leads to fewer unit operations as compared to biorefining strategies based on bacterial fermentations. The direct assimilation of cereal grains by fungi alleviates the need for addition of complex nutrient supplements, such as yeast extracts, because cereal grains contain all the necessary nutrients for microbial growth. In addition, fungi produce enzymes that could be either separated as a value-added coproduct or reused to enhance the hydrolysis of cereal components.

At the Satake Centre for Grain Process Engineering (SCGPE), we target the development of sustainable cereal-based biorefineries. We have published various biorefining strategies based on wheat for the production of fuels, platform chemicals, and biodegradable plastics (*8*−*10*). Aiming at the development of tailor-made biorefineries depending on the properties of the selected cereal crop, another biorefining strategy based on oats was recently proposed (*11*). In this study, we evaluated an oat-based biorefining scheme for the production of lactic acid as well as other value-added byproducts, such as β-glucan and antioxidant-rich oil bodies (**Figure 1**). The process begins with pearling of oat groats to produce bran-rich pearlings and endosperm-rich pearled oat groats. After air classification of oat pearlings, the remaining bran-rich fraction could be used for the extraction of a spectrum of value-added coproducts (*12*): a highly concentrated anti-irritant and a light oat oil extracted successively by a volatile aqueous polar solvent and a nonpolar solvent; a dark high quality oil and a highly stable

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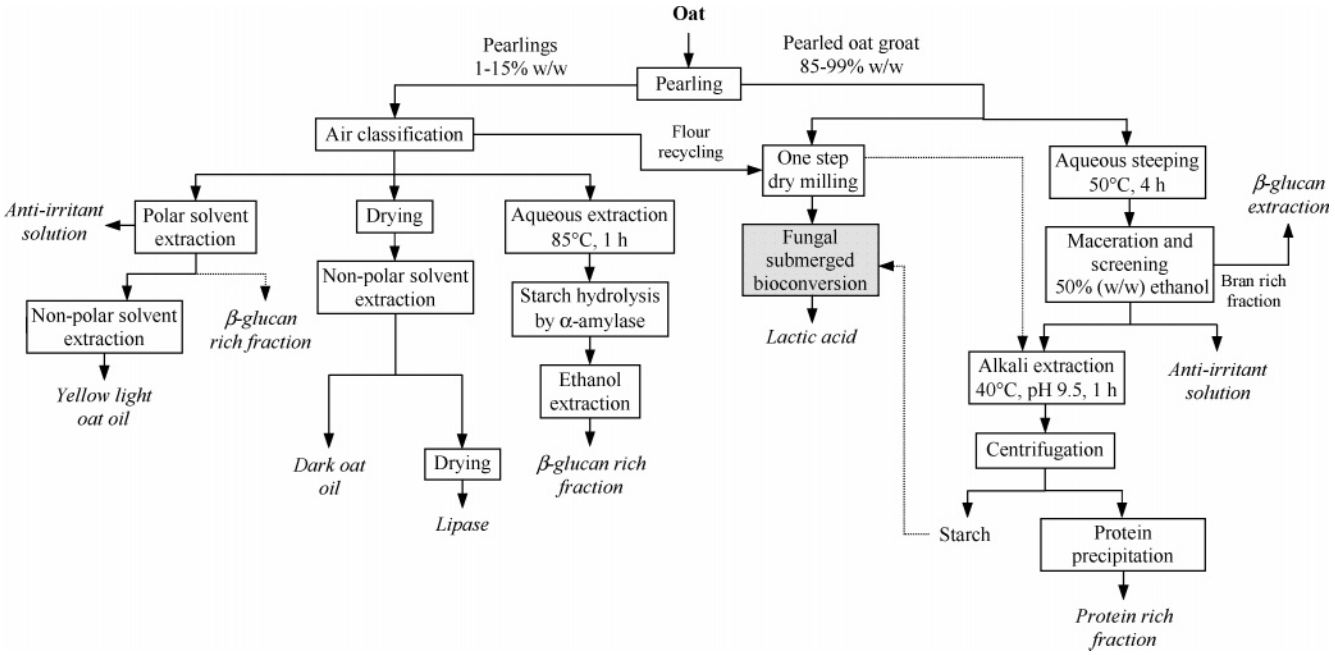

Figure 1. Proposed oat-based biorefinery for the production of lactic acid and various value-added coproducts.

lipase-active powder extracted by a nonpolar solvent; and a β-glucan rich fraction extracted by successive aqueous steeping of bran, starch removal by enzymatic hydrolysis, bran removal by screening/centrifugation, and alcoholic extraction.

The majority of the initial oat kernel (at least 85% of the whole) remains after pearling and air classification as an endosperm-rich fraction. Flour from pearled oat kernels can be used directly as the sole medium in fungal bioconversions for the production of lactic acid. It has been reported that lactic acid can be produced at high quantities (102 g/L lactic acid from 120 g/L starch) in airlift bioreactors from starch, ammonium sulfate, and small quantities of mineral salts (*5*). This provides the ability to fractionate the endosperm-rich pearled oat grains into starch that can be used in fungal lactic acid fermentations as well as some value-added coproducts, such as protein, anti-irritant solution, and β-glucan (**Figure 1**).

This study presents the potential of *Rhizopus oryzae* fermentation in a bioreactor on various concentrations of pearled oat flour for the production of lactic acid. In most previous studies, the carbon source that was used in fungal fermentations for lactic acid production was either glucose (*13*, *14*) or liquefied/saccharified starch solutions (*5*,*15*−*18*). The nitrogen sources used in those studies were mainly inorganic chemicals or byproducts of food industries. Previous studies presenting kinetics of fungal lactic acid fermentations on whole cereal flour were limited to shake flasks (*19*, *20*). The development of cost-competitive lactic acid production would be achieved by using whole crops with optimal use of starch and protein, while the rest of the components in cereals will be used for the production of value-added coproducts. In addition, *R. oryzae* produces enzymes during fermentation and this study stresses their importance in the proposed oat-based biorefining strategy.

MATERIALS AND METHODS

Microorganism. The strain *Rhizopus oryzae* NRRL 395 (Northern Regional Research Center, USDA-ARS, Peoria, IL) was used in fungal fermentations for lactic acid production. It was activated in nutrient broth at 30 °C for 2 days before being transferred onto slopes containing solid medium made of 5% (w/v, on a dry basis, db) pearled oat flour and 2% agar. Spores produced after 7 days incubation at 30 °C were stored at 4 °C. One slope was used as fermentation inoculum.

Table 1. Composition of Whole Oat Groats and Oat Fractions Produced by Pearling

components	weight (%)		
	whole oat groats	pearlings	pearled oat flour
moisture (wet basis)	13.4 ± 0.05	3.1 ± 0.04	13.3 ± 0.05
ash (dry basis)	1.6 ± 0.01	11.7 ± 0.01	1.45 ± 0.02
starch (dry basis)	63.7 ± 1.2	0.4 ± 1.1	65.2 ± 0.91
protein (dry basis)	10.4 ± 0.09	2.1 ± 0.07	10.45 ± 0.08
phosphorus (dry basis)	0.37 ± 0.01	6.46 ± 0.02	0.36 ± 0.01
potassium (dry basis)	0.37 ± 0.03	10.86 ± 0.01	0.34 ± 0.03
magnesium (dry basis)	0.15 ± 0.02	8.84 ± 0.01	0.14 ± 0.02

Fungal Bioconversions. An oat variety of *Expression*, supplied by Oat Services Ltd. (United Kingdom) from the harvest in 2002, was used as the sole nutrient source in *R. oryzae* bioconversions. Oat kernels were pearled for 20 s for the removal of 2.5% (w/w, db) pearlings using a Satake Abrasive Test Mill (model TM05, Satake Corporation, Hiroshima, Japan) with a batch size of 220 g groats. An abrasive wheel with a mesh size of 40 was used. The essential parts of this mill include an abrasive wheel, driven by a motor at 1450 rpm, and a screen with slots of 1.0 mm in width housing the wheel. Four baffles were fixed onto the internal surface of the screen to enforce turbulent movement of the grains along the gap between the wheel and the screen. The gap measured 16 mm in depth and the baffles 3 mm in thickness. During operation, the abrasion between the wheel surface and the groats detached bran sections (hereafter referred to as pearlings) from the groat surface. The centrifugal force generated by the rotating wheel serves to discharge the pearlings through the slots on the screen. Pearled grains were milled into flour (**Table 1**) using a hammer mill (Falling Number AB) with a screen size of 500 μm.

Fungal fermentations were conducted in a 10-L bioreactor with 7-L working volume. Fermentation temperature was controlled at 30 °C and pH at 6 with 10 M NaOH. Aeration rate and agitation speed varied between 1 and 3 vvm and between 250 and 350 rpm, respectively. The initial fungal spore concentration was 2×10^6 spores mL^{-1}. Detailed description of the bioreactor used is presented in a previous publication (*9*). Fermentation media were sterilized for 4 h at 121 °C either in a 10-L vessel and then transferred into the bioreactor or inside the bioreactor when pearled oat flour concentrations higher than 100 g/L were used. In-situ sterilization of flour suspension was carried out because it was impossible to pump a medium of high flour concentration into the bioreactor when it was sterilized separately.

When flour concentrations higher than 100 g/L were used, fermentation pH was maintained at 5 until lactic acid production started to prevent bacterial contamination. It has been stressed in the literature (*5*) that *R. oryzae* cultivations at high starch concentrations may result in bacterial contamination. However, this study demonstrates that lactic acid fermentation on high flour concentrations is feasible when a lower pH value than the optimum was used until lactic acid production began. After this point, fermentation pH was maintained at 6 and no bacterial contamination was observed. No bacterial contamination was observed at fermentations carried out with flour concentrations up to 66.3 g/L when a pH value of 6 was used throughout those experiments.

Fermentations on varying oat flour concentrations (29.7, 40.3, 44.5, 56.5, 66.3, 100.5, 116.5, and 152 g/L) were carried out to investigate the production of L-lactic acid by *R. oryzae*. Fermentations with flour concentrations between 29.7 and 66.3 g/L were carried out at 1 vvm aeration rate and 250 rpm agitation speed. Fermentations at higher flour concentrations (100.5−152 g/L) were carried out with increased aeration rate (2 and 3 vvm) and the same or higher agitation speed (250 and 350 rpm) to investigate whether lactic acid production and starch to lactic acid conversion rates could be simultaneously increased. All fermentations were carried out twice. The second fermentation for each initial oat flour concentration was carried out to verify the maximum lactic acid concentration and starch to lactic acid conversion yield. Samples from repeated experiments were only taken toward the end of the fermentations. The difference of maximum lactic acid production and starch to lactic acid conversion yield between the two fermentations conducted for each pearled oat flour concentration was less than 7%, apart from fermentation on 44.5 g/L flour where the difference was 11%. Fermentation duration was the same for both experiments conducted for each flour concentration.

Analytical Methods. Whole oat groats, pearlings, and pearled oat grains were analyzed in terms of moisture, starch, ash, protein (on the basis of total Kjeldahl nitrogen (TKN) analysis), phosphorus, potassium, and magnesium. The moisture content in oat flours was analyzed by drying three samples of preweighed oat flour (around 1 g) at 105 °C for 24 h. After drying, the samples were transferred to a desiccator to cool for 2 h before being weighed. The starch content in oat flours was analyzed by an enzymatic starch analysis kit (Megazyme International, Ireland, www.megazyme.com). TKN was analyzed by the Nessler spectrophotometric method (*21*). Phosphorus, potassium, and magnesium in oat flour were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Vista MPX, Varian, Inc.) after complete digestion of three preweighed (around 1 g) flour samples with 4 mL H_2SO_4 at 440 °C in 100-mL Digesdahl digestion flasks (Hach).

Samples (10 mL) of fermentation broth were regularly removed from the bioreactor for the determinations of total dry weight, lactic acid, glucose, free amino nitrogen, and phosphorus. Each sample was centrifuged at 3000*g* for 10 min. The sediment was used for the determination of dry weight change throughout fermentation. Glucose and lactic acid concentrations were analyzed using an Analox GL6 analyzer. Only L(+)-lactic acid was produced. Free amino nitrogen (FAN) concentration was analyzed by the ninhydrin colorimetric method (*22*). Phosphorus in liquid samples was analyzed by the method described by Herbert et al. (*23*).

Enzyme Activity Assays. Enzyme activities of glucoamylase, protease, and phosphatase produced during *R. oryzae* fermentations on pearled oat flour were analyzed by a novel enzymatic assay developed at the SCGPE (*24*). This method utilizes cereal flour (oat flour in the current study) as the substrate for measuring simultaneously the activity of all the enzymes involved in the hydrolysis of major cereal compounds (starch, protein, and phytic acid). The enzymes analyzed represent groups of enzymes that hydrolyze the same type of macromolecule.

A pearled oat flour suspension of 5% (w/v, db) was prepared in a 250-mL Duran bottle and then was gelatinized at 70 °C for approximately 10 min to decrease the hydrolytic resistance of starch. Two milliliters of the gelatinized flour suspension was transferred into a 14-mL sample test tube and was placed in a water bath set at 55 °C. The pH of the reaction mixture was not controlled but was measured as about 5.5. Then, 2 mL of fermentation supernatant was transferred into each centrifuge tube. The entire sample was then shaken and 0.5 mL of the sample was transferred to a microcentrifuge tube containing 0.5 mL of frozen 5% (w/v) trichloroacetic acid that stopped the enzymatic reaction. Samples were taken at various time intervals depending on the activity of the enzymes analyzed. In this way, initial rate reactions were calculated for all enzymes. The components analyzed for the measurement of the activities of glucoamylase, protease, and phosphatase were glucose, FAN, and phosphorus, respectively. All enzymes were expressed in U/mL. In the case of glucoamylase, one unit (U) is defined as the amount of enzyme required to produce 1 mg of glucose per min of reaction under the assay conditions. In the case of protease and phosphatase, one unit is defined as the amount of enzyme required to produce 1 μg of FAN or phosphorus, respectively, per min of reaction under the assay conditions.

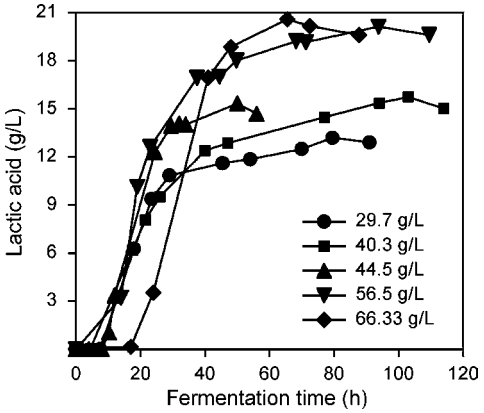

Figure 2. Profile change of lactic acid concentration during *R. oryzae* fermentations conducted at 250 rpm agitation speed and 1 vvm aeration rate on five initial pearled oat flour concentrations (29.7, 40.3, 44.5, 56.5, and 66.3 g/L).

Statistical Analysis. Multiple ordinary least-square (OLS) regression was carried out in SPSS with response variable the starch to lactic acid conversion and explanatory variables the initial flour concentration and aeration rate.

RESULTS AND DISCUSSION

Lactic Acid Production during Fermentation on Pearled Oat Flour. Figure 2 presents the lactic acid production during five fermentations on varying pearled oat flour concentration (29.7, 40.3, 44.5, 56.5, and 66.3 g/L). The duration of each one of these fermentations was varied to ensure that the highest lactic acid concentration had been achieved. The production of lactic acid clearly demonstrated that higher flour concentration led to higher lactic acid production. The highest lactic acid concentration (20.6 ± 0.49 g/L) was achieved with a pearled oat flour concentration of 66.3 g/L, which corresponds to a starch to lactic acid conversion ratio of 0.48 g/g, after a fermentation time of 65 h. These results suggested that the production of lactic acid could be further increased if an even more concentrated medium was used.

The increase in flour concentration led to a decrease in the conversion ratio from starch to lactic acid (**Figure 3**). This was probably caused because of the lack of dissolved oxygen, which in all fermentations carried out in this study follows the trend shown in **Figure 4**. During the exponential growth phase, dissolved oxygen concentration falls below 5% of saturation, which is lower than the critical level of dissolved oxygen for most industrial fungi, such as *Aspergillus* species (*25*). The lack of dissolved oxygen has also been indicated in other studies (*16*, *26*) as the main reason for reduced starch to lactic acid conversion rates. Skory et al. (*26*) claimed that under oxygen-limiting conditions pyruvate is metabolized to ethanol instead of lactic acid. Miura et al. (*16*) reported that lactic acid

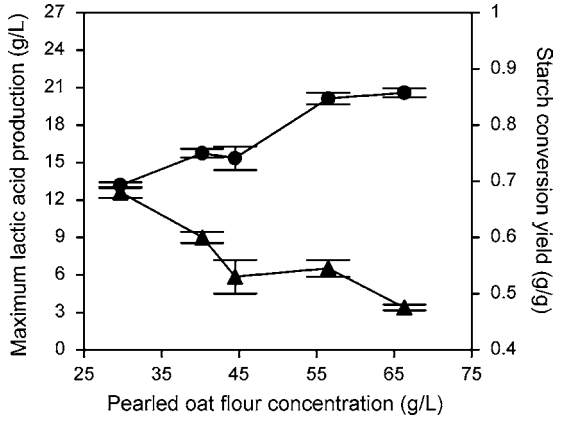

Figure 3. Effect of initial pearled oat flour concentration on maximum lactic acid production (●) and starch to lactic acid conversion yield (▲).

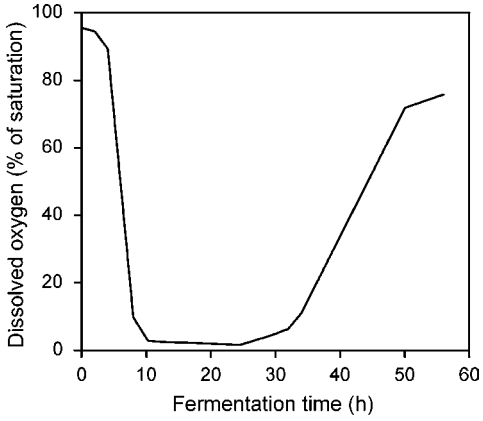

Figure 4. Representative profile change of dissolved oxygen concentration during *R. oryzae* fermentation on pearled oat flour.

production rates and yields in 100-L airlift bioreactors were dependent on superficial air velocity (V_s) and volumetric oxygen transfer rate coefficient (K_La).

Direct measurement of fungal growth was not possible because of the presence of solid particles throughout fermentation. The growing fungus adhered to solid particles (especially oat bran), and their separation was impossible. This resulted in a highly dispersed morphology and a viscous broth throughout fermentation that may have reduced oxygen transfer. The trend of fungal growth can only be assumed by taking into consideration the consumption of dissolved oxygen (**Figure 4**). The duration of the phase where the dissolved oxygen level is below 5% of saturation was prolonged depending on the initial flour concentration until all starch had been consumed. A linear fungal growth should be expected during this phase.

Figure 5 presents representative profile changes of glucose, FAN, and phosphorus during *R. oryzae* fermentations carried out on 29.7 and 44.5 g/L pearled oat flour. These results indicate that *R. oryzae* produces amylolytic, proteolytic, and phosphorus-producing enzymes to obtain directly assimilable carbohydrates, amino acids, peptides, and phosphorus from oat macromolecules (starch, protein, phytic acid). In the early fermentation stage (0−20 h), a slight increase in glucose concentration was observed (**Figure 5a**) obviously owing to the production of amylolytic enzymes. When the cells entered their exponential growth phase, a sharp decrease in glucose concentration occurred. The limited amylolytic activity only managed to maintain glucose concentration at an average of about 0.3 g/L throughout the later fermentation stage. The profile of glucose actually represents the dynamic equilibrium between starch hydrolysis and glucose consumption. In the early stage of fermentation, the high concentration of starch and the low concentration of fungal cells ensured that the rate of glucose production was higher than that of glucose consumption, resulting in the continuous increase in glucose concentration, although amylolytic activity at this stage was undoubtedly lower than that at the later stage. During the later stage, the decrease in starch concentration and the increase in glucose consumption because of cell growth caused glucose consumption to exceed glucose production, leading to the reduction of glucose concentration.

The trend of the profile change of FAN concentration (**Figure 5b**) throughout these fermentations was similar to glucose. An initial increase in FAN concentration was followed by a sharp decrease to a value of around 8 mg/L. This constant FAN concentration that was observed toward the end of fungal fermentations was increased up to 18 mg/L with increasing pearled oat flour concentration. Possible explanations for the residual FAN concentration toward the end of fermentations might include (1) the digestible carbon to nitrogen ratio in pearled oat flour was slightly lower than the requirement by *R. oryzae*; (2) the fungus did not produce sufficient protease activity for the complete hydrolysis of oat protein into amino acids, which means that the residual FAN concentration reflects the content of protein and peptones in the broth; and (3) some amino acids from oat protein are not essential nitrogen sources for *R. oryzae*.

In the case of phosphorus, a different trend was observed, as it was decreased continuously during fermentation to a value around 10 mg/L (**Figure 5c**). This indicated either the production of insufficient amount of phosphatase required to release all phosphorus present in oat flour or an extremely high phosphorus consumption rate by *R. oryzae*. The slight recovery in the phosphorus profiles toward the end of fermentation was most likely the result of cell autolysis (*27*). Phosphorus is one of the most important minor nutrients for the majority of microorganisms. Over 80% of the total phosphorus in cereal flour is in the aleurone cells in the form of phytic acid (*28*). In cereal flour media, fungal cells obtain phosphorus mainly from phytic acid by producing phytase (*29*). By using the general term phosphatase, we refer to the group of enzymes required to release phosphorus from oat components including phytic acid. The access to phytic acid and other phosphorus-containing oat components will also depend on the production of other enzymes required for the hydrolysis of aleurone cell wall and other layers in oat kernel that may not be produced by *R. oryzae*.

Fungal fermentations of oat flour concentration higher than 66.3 g/L were carried out at higher aeration rate and agitation speed to investigate the effect of oxygen transfer in the broth on lactic acid production and starch to lactic acid conversion yield. Three fermentations were conduced at 100.5, 116.5, and 152 g/L pearled oat flour (**Figure 6**) using the same conditions as in the fermentations presented in **Figure 2** apart from aeration rate which was set at 2 vvm. The fermentations presented in **Figure 6** demonstrated that better oxygen transfer increases lactic acid production and starch to lactic acid conversion yields. A 100.5 g/L (db) pearled oat flour concentration and an aeration rate of 2 vvm resulted in a maximum starch to lactic acid conversion ratio of 0.65 ± 0.016 g/g, which is similar to the level achieved when a flour concentration of 40.3 g/L was used (**Figure 2**). The use of 116.5 g/L pearled oat flour and 2 vvm aeration rate resulted in higher lactic acid production (47.9 ± 1 g/L) and slightly lower starch to lactic acid conversion yield (0.63 g/g). At the same agitation speed and aeration rate, a

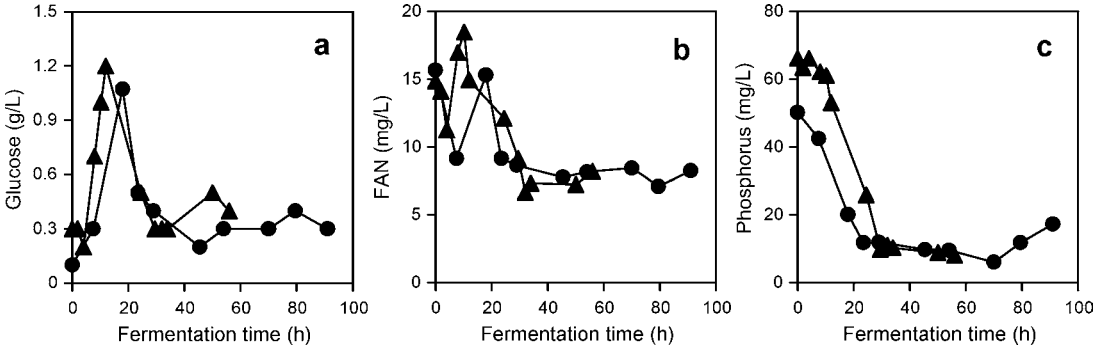

Figure 5. Profile change of glucose, FAN, and phosphorus concentration during *R. oryzae* fermentation on 29.7 g/L (●) and 44.5 g/L (▲) pearled oat flour.

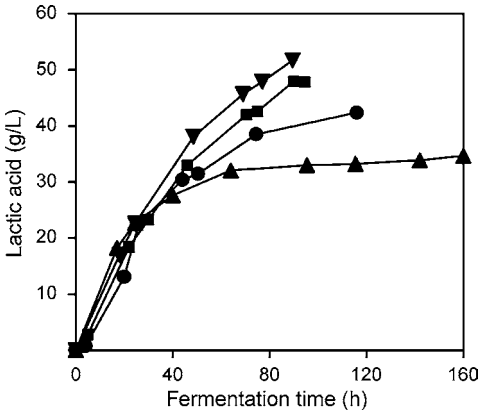

Figure 6. Profile change of lactic acid concentration during *R. oryzae* fermentation conducted on three initial pearled oat flour concentrations at two agitation speeds and aeration rates. (●), 100.5 g/L, 250 rpm, 2 vvm; (■), 116.5 g/L, 250 rpm, 2 vvm; (▲), 152 g/L, 250 rpm, 2 vvm; (▼), 116.5 g/L, 350 rpm, 3 vvm.

significant decrease in lactic acid production (34.6 ± 1.8 g/L) was observed when a higher flour concentration (152.05 g/L) was used. This most probably occurred because of the higher broth viscosity that led to reduced oxygen transfer. Miura et al. (*16*) also reported that lactic acid production rate and yield from liquefied starch suspensions decreased significantly when starch concentration (expressed as glucose) was increased from 120 (0.86 g/g and 2.51 g/L/h) to 150 g/L (0.77 g/g and 1.98 g/L/h). Yin et al. (*5*) stressed that liquefied starch concentrations higher that 120 g/L resulted in lower lactic acid production and yield.

The effect of initial flour concentration (29.7, 40.3, 44.5, 56.5, 66.3, 100.5, 116.5, and 152 g/L) and aeration rate (1 and 2 vvm) on starch to lactic acid conversion yield has been statistically described by multiple OLS regression. The resulting regression equation (conversion = 83.66 − 0.57 × flour + 40.58 × aeration) explains 89.7% of the variance of the dependent variable ($R^2 = 0.897$). According to the model, initial flour concentration and aeration rate have negative and positive effects on the conversion, respectively. In particular, when the aeration rate changes from 1 to 2 vvm, the measure of conversion increases by an average of 40.58 (according to the dummy coding of this categorical variable). Both flour concentration and aeration rate have a statistically significant effect ($p < 0.01$).

Lactic acid production (51.7 ± 1.03 g/L) and starch to lactic acid conversion yield (0.68 ± 0.013 g/g) during fermentation of 116.5 g/L flour concentration (**Figure 6**) were further improved when agitation and aeration rate were increased to 350 rpm and 3 vvm, respectively. The starch conversion yields achieved in this fermentation was approximately 3 times higher than the one achieved by Yu and Hang (*19*) that reported a lactic acid production yield of 23% on the basis of the amount of carbohydrates as glucose that was consumed. Those experiments were carried out in shake flask using a 10% (w/v, on a dry basis) whole oat flour suspension at 30 °C for 72 h. It has been reported that the use of oats in *R. oryzae* fermentations resulted in the lowest lactic acid production yield as compared to corn, barley, and rice (*19*, *20*, *30*). However, an advantage of oats as compared to other cereal crops is that it has no major applications in the food industry and contains several interesting components, such as antioxidants and β-glucan, which could form high-value coproducts alongside starch fermentation within an integrated biorefinery.

Preliminary results from *R. oryzae* fermentations on pearled oat flour presented in this study showed that the biorefining strategy proposed in **Figure 1** could be gradually developed into a viable industrial process. Significant cost reduction as compared to bacterial lactic acid fermentations will be achieved by avoiding expensive starch hydrolysis and purchase of a spectrum of enzymes required to hydrolyze oat macromolecules.

Process economics could also be improved by using pearling of oat groats. This fractionation technology does not only produce pearlings that can be used for the extraction of value-added coproducts but also reduces the microbial population present in cereal grains (*31*). Microbial contaminants are present within a certain distance from the surface of cereal grains. Laca et al. (*31*) reported that removing 4% of the total grain weight by pearling would result in the reduction of the microbial load in cereal grains by approximately 87%. Using aseptic conditions during pearling may result in a flour suspension of low microbial load and therefore reduced sterilization requirements prior to fermentation.

Higher lactic acid production and starch to lactic acid conversion yields could be achieved by increasing oxygen transfer rates. This could be accomplished by increasing aeration rate and agitation speed or using an alternative bioreactor design, such as airlift bioreactors. Yin et al. (*5*) reported that the use of an airlift bioreactor resulted in higher lactic acid concentration, productivity, and yield when compared to a conventional jar bioreactor. Significant growth on most parts of the stirred-tank reactor should result in reduction of oxygen transfer to the aggregated hyphae.

Enzyme Production during Fermentation of *R. oryzae* on Pearled Oat Flour. The growth of *R. oryzae* during submerged fermentation on pearled oat flour indicated that a range of enzymes were produced, which are required to hydrolyze oat macromolecules into directly assimilable monomers. Preliminary analysis of enzyme activities was carried out using oat pearled flour as a universal substrate for measuring the hydrolytic activity of amylolytic, proteolytic, and phosphorus-releasing

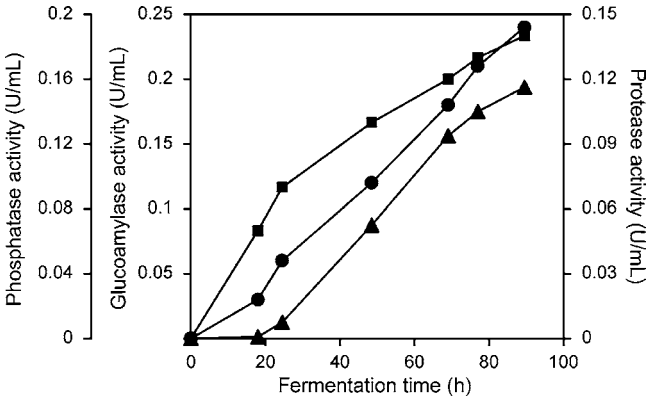

Figure 7. Profile change of glucoamylase (●), protease (■), and phosphatase (▲) activities during *R. oryzae* fermentation on 116.5 g/L pearled oat flour at 3 vvm aeration rate and 350 rpm agitation speed.

enzymes. Amylolytic enzymes were described in this study as glucoamylase because the product of starch hydrolysis measured was glucose. **Figure 7** presents profiles of glucoamylase, protease, and phosphatase activity throughout fermentation of 116.5 g/L pearled oat flour with an agitation speed of 350 rpm and aeration rate of 3 vvm. Amylolytic activity was sufficient to hydrolyze starch contained in all pearled oat flour concentrations used. This was justified by conducting starch analysis of the remaining solids after the end of fungal fermentations.

Proteolytic activity was high enough to result in the accumulation of FAN in the broth in the early fermentation stage (**Figure 5b**). Toward the end of *R. oryzae* fermentation, both FAN and phosphorus concentration reached approximately constant values indicating that protease and phosphatase activities produced during fermentation were sufficient to release surplus FAN and phosphorus for the exhaustion of the starch content by fungal cells. This also means that pearled oat flour contained sufficient nutrients for the exhaustion of all glucose hydrolyzed from starch by the fungus.

Fungal growth and lactic acid formation could be accelerated by providing directly assimilable monomers to the fungal cells. This will also lead to lower broth viscosity and higher oxygen transfer rate. It has been reported that the use of glucose may result in higher yields and lactic acid concentrations as compared to unhydrolyzed or liquefied starch (*32*). It is envisaged that hydrolysis of oat macromolecules could be accomplished by recirculating broth filtrate to be mixed with pearled oat flour prior to fermentation. Heating this flour suspension up to 70 °C would result in the dextrinization of starch as well as protein and phytic acid hydrolysis. This processing strategy will be tested in future work. A similar processing technique has been proposed for the hydrolysis of wheat flour by a crude fermentation filtrate containing a consortium of enzymes produced via *Aspergillus awamori* fermentation of wheat flour (*9*, *24*). In addition, the presence of lactic acid combined with the reduction of microbial contaminants by pearling of oat groats may prevent sterilization of flour suspensions prior to fermentations. This processing strategy will also result in increased flour concentration to the bioreactor and possibly to lactic acid production.

To increase the amount of nutrients in initial fermentation broth, autolysis of remaining fungal biomass would result in the regeneration of many essential nutrients consumed during fermentation. Autolysis of fungal cells produced during fermentation has been developed as a means to minimize waste disposal and to reduce the loss of nutrients contained in the initial grain (*27*). Autolytic experiments carried out with remaining solids from *R. oryzae* fermentations (results not shown) were very encouraging.

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