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Utilisation of waste bread for fermentative succinic acid production

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ABSTRACT

A novel biorefinery concept of utilising waste bread as a sole nutrient source for the production of a nutrient rich feedstock for the fermentative succinic acid production by *Actinobacillus succinogenes* has been developed. Waste bread was used in the solid-state fermentations of *Aspergillus awamori* and *Aspergillus oryzae* that produce enzyme complexes rich in amylolytic and proteolytic enzymes, respectively. The resulting fermentation solids were added directly to a bread suspension to generate a hydrolysate containing over 100 g/L glucose and 490 mg/L free amino nitrogen (FAN). A first-order kinetic model was used to describe the effect of initial bread mass ratio on glucose and FAN profiles. The bread hydrolysate was used as the sole feedstock for *A. succinogenes* fermentations, which led to the production of 47.3 g/L succinic acid with a yield and productivity of 1.16 g SA/g glucose and 1.12 g/L h. This corresponds to an overall yield of 0.55 g succinic acid per g bread. This is the highest succinic acid yield compared from other food waste-derived media reported to date. The proposed process could be potentially utilised to transform no-value food waste into succinic acid, one of the future platform chemicals of a sustainable chemical industry.

1. Introduction

Food waste is one of the largest potions of municipal solid waste with a fraction falls between 12% and 39% among different countries, such as United States [1], Hong Kong [2] and Singapore [3], which causes severe environmental problems. Utilisation of food waste as the renewable feedstock in biorefinery for chemicals and energy production becomes a promising option to target both solid waste problem and the over-dependence on petroleum as the major source of chemicals and energy [4,5]. The nutrients contained within food waste can be converted by micro-organisms into desired products, such as biofuels [6–8], functional chemicals [9] and monomers of bioplastics [10].

Succinic acid (SA), as a precursor for many chemical substances [11] with a production capacity of 30,000 tonnes per year and a corresponding market value of $225 million [12], has attracted research interest in the development of fermentative SA production as an alternative to the current non-sustainable petrochemical route. This bio-based SA production has a lower energy consumption due to milder operation conditions and lower dependence on a single feedstock [13,14]. The common micro-organisms used in fermentative SA bio-production are *Actinobacillus succinogenes* [15], *Anaerobiospirillum succiniciproducens* [16], *Mannheimia succiniciproducens* [17] and recombinant *Escherichia coli* [18]. The possibility of using these micro-organisms to convert food waste into SA has been explored. Yu et al. [19] examined SA production from corncob by *A. succinogenes*. Li et al. [20] demonstrated SA production from orange peel by *Fibrobacter succinogenes*.

Nutrients in food are stored in the form of macromolecules such as starch and proteins. To facilitate the growth of micro-organisms, these large molecules have to be broken down into utilisable form such as sugars and amino acids. Usually, enzymatic hydrolysis using α-amylase and protease can efficiently facilitate this process [21]. Compared with methods for the hydrolysis of lignocellulosic based materials [22], enzymatic hydrolysis has several outstanding advantages. This includes mild reaction conditions, avoidance of using hazardous chemicals and reduced risks of generating fermentative inhibitors. Apart from using commercial available enzymes in the hydrolysis, solid-state fermentation [23] is a preferred option to produce enzymes with advantages such as high enzyme concentration due to absence of free water and low protein breakdown [24].

Among various micro-organisms used in solid state fermentations (SSF), filamentous fungi are widely exploited. Du et al. [25] developed a strategy of generating glucoamylase from *Aspergillus awamori* and protease from *Aspergillus oryzae* in SSF on wheat.

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Nomenclature

A	linear regression constant for Eq. (4) (g/L)
B	linear regression constant for Eq. (4) (g/L)
$C(t)$	glucose/FAN concentrations at time t (g/L for glucose; mg/L for FAN)
C_∞	saturation concentration of glucose/FAN (g/L for glucose; mg/L for FAN)
k	kinetic constant (h^{-1}).
FAN	free amino nitrogen
OD_{660}	optical density at 660 nm
SA	succinic acid
SSF	solid-state fermentation
t	time (h)
TS	total sugars (g/L)
x	initial bread mass ratio (w/v%)

These enzymes were utilised to hydrolyse gluten-free flour and gluten into carbon-rich and nitrogen-rich streams, respectively. Dorado et al. [26] devised another strategy of combining hydrolysis of starch and protein with fungal autolysis. In this strategy, *A. awamori* and *A. oryzae* SSF solids were added to wheat middlings and bran at 55 °C to produce a nutrient-complete stream, which was subsequently utilised for fermentative SA production. Melikoglu [27] developed a multi-enzyme solution of glucoamylase and protease during solid-state fermentation using *A. awamori*. This was used in subsequent hydrolysis reactions to produce nutrient rich hydrolysates from waste bread pieces and from wheat flour. Considering the similar composition of waste bread and wheat, it should be possible to utilise waste bread as a feedstock for the production of fermentation medium using a novel bioprocess as illustrated in Fig. 1.

2. Materials and methods

All chemicals involved in this study were obtained from Sigma–Aldrich, US except otherwise specified.

2.1. Micro-organisms

A. awamori ATCC 14331 was used for the production of amylolytic enzymes. An industrial strain of *A. oryzae* isolated from a soy sauce starter kindly provided by the Amoy Food Ltd., Hong Kong. It was utilised to produce proteolytic enzymes. Their storage and sporulation for inoculum preparation have been described in a previous publication [25]. *A. succinogenes* ATCC 55618 were utilised for succinic acid fermentations.

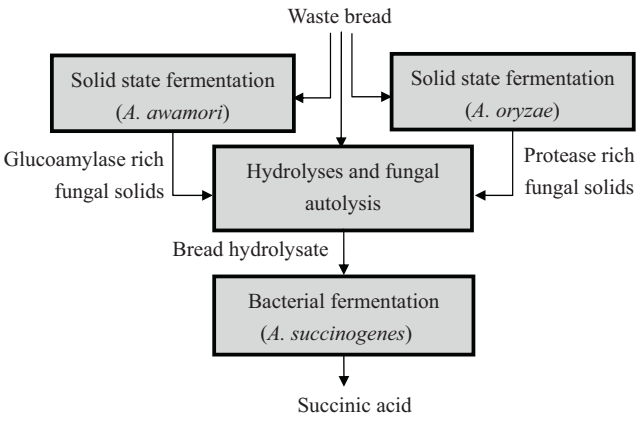

Fig. 1. Process flow diagram of the proposed waste bread biorefinery concept for succinic acid production. The biorefinery consists of three steps: (1) *A. awamori* and *A. oryzae* SSF were carried out on waste bread to obtain glucoamylase and protease-rich fungal solids. (2) The solids were added to a waste bread suspension to produce a nutrient-rich hydrolysate. (3) *A. succinogenes* fermentation using the waste bread hydrolysate for SA production.

2.2. Raw materials

Waste bread was kindly provided by the Coffee Shop at the Hong Kong University of Science and Technology (HKUST). Moisture and starch were analysed according to procedures described in Koutinas et al. [28].

2.3. Solid state fermentation (SSF)

Bread was cut into cubes of approximately 1 cm^3 in size and autoclaved for 15 min prior to cultivation. Ten grams of bread cubes were transferred into each Petri dish. One millilitre of cryopreserved spores of *A. awamori* (2.85×10^7 spores/mL) or *A. oryzae* (6.31×10^6 spores/mL) were diluted by 10-folds and spread evenly on bread pieces separately. The prepared samples were incubated at 30 °C for 4 days.

2.4. Enzymatic hydrolysis

Two 2.5 L bioreactors equipped with automatic temperature control water jacket and stirrers were used for enzymatic hydrolysis. Various amount of waste bread pieces were blended with 1 L water for 15 min. The resulting mixture was transferred into the bioreactors at 55 °C. Fungal marshes from SSF were added into the vessel. The reaction mixture pH was not controlled and was stirred at 300 rpm. Samples were taken every hour for 24 h. The resultant broth was centrifuged at 10,000 rpm for 15 min. The supernatant was subsequently filtered by vacuum filtration using Whatman No.1 filter paper. All experiments were carried out in duplicate.

2.5. Bacterial fermentation

Two 2.5 L fermentors (Biostat, Germany) were used for fermentative SA production by *A. succinogenes*. Bacterial fermentation using bread hydrolysate was preformed and the inoculums size was 5%v/v. The initial glucose concentration of the broth was 40 g/L while the free amino nitrogen (FAN) concentration was 200 mg/L. The latter corresponds to the FAN content of a 4 g/L yeast extract solution. The hydrolysate was filtered by 0.2 μm PTFE membrane filter (Sartorius, Germany). Prior to autoclaving, 10 g/L magnesium carbonate ($MgCO_3$) was added to the fermentation medium as a neutral pH buffer. The pH of the fermentation broth was automatically controlled within 6.6–6.8 with the addition of 10 M NaOH and 0.05 M H_2SO_4. The broth was sparged with 0.5 vvm CO_2 and agitated at 300 rpm. Fermentation samples were taken every 3 h to measure optical density and metabolites concentration. Fermentations were considered to have ended when glucose was completely depleted.

2.6. Analytical techniques

2.6.1. Cell density measurement

Bacterial growth was determined by optical density (OD) measurements at 660 nm (spectrophotometer UV-1800, Shimadzu, Japan). Optical density (OD), glucose and fermentation metabolites were determined as described previously [17]. At an OD_{660} of 1.0 in TSB, *A. succinogenes* has a concentration of 0.626 g dry cell weight (DCW)/L [17].