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Utilization of Wheat Biorefining Strategy Based on Solid-State Fermentation for Fermentative Production Technique

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ABSTRACT

In this study, the development of a new generic feedstock manufacturing technique based on SSF, which was then applied to the succinic acid fermentation process. Separation of the wheat was accomplished using milling and gluten extraction techniques. This waste product from wheat milling, known as bran, was used to manufacture glucoamylase and protease enzymes using Aspergillus oryzae and Aspergillus awamori. A total of 140 g l 1 glucose and more than 3.5 g l 1 free amino nitrogen were produced after hydrolysis of both the gluten-free and gluten-containing solutions. These two sources, when combined, supplied all the nutrients required for succinic acid fermentation mediated by Actinobacillus succinogenes. A fermentation employing just the mixed hydrolysate streams yielded about 22 g l 1 succinic acid. With the addition of $MgCO_3$ to the wheat-derived medium, succinic acid production rose to 64 g l 1. Using the SSF-based technology, it was possible to create a generic wheat feedstock that could be utilised to make succinic acid and then demonstrate the effectiveness of the proposed methodology.

1. INTRODUCTION

There should be more sustainable ways to produce new goods (food and non-food, chemicals) from existing food and agro-industrial waste than what is now possible. A high cost of raw materials is indicated by the use of commercial enzymes that are expensive. While the second approach relies on bacteria that can break down starch, it has been employed only a few times, the most common of which being lactic acid and bioethanol fermentations [1]. A sustainable approach to production entails making effective use of resources, creating little waste and/or low-value streams, and providing consumers with items and/or materials that meet their needs in terms of functionality, safety, and integrity. Such biotechnology-based goods may be produced using SSF and similar technologies instead of traditional methods [2]. Polymer manufacturing relies heavily on ancient hydrocarbons for its current technology. New sustainable energy sources based on renewable raw materials are currently necessary because of the increasing demand for energy, the decreasing availability of fossil fuels, and the growing awareness of environmental concerns [3]. For this reason, it is critical to choose acceptable raw materials and create biorefinery-based technologies that enable long-term processes. Cereals (particularly wheat) have potential as sustainable raw materials in the United Kingdom [4]. When it comes to bacterial fermentations, wheat has all the nutrients required, but bacteria can't get to them since they are inaccessible to them [5]. In order to unleash these nutrients, some kind of preprocessing is required [6]. It is possible to make platform chemicals in a number of ways, but the most typical ones are as follows:

(1) Making fine compounds from glucose liberated during the processing of wheat into reasonably pure starch.

(2) Using particular types of microorganisms in conjunction with wheat flour.

(3) Investigating a biorefining technique that converts wheat's whole nutritional content into a generic feedstock, which is then fermented to produce fine compounds from?

However, the first strategy relies only on starch, with little consideration given to the other wheat-derived compounds [7]. Using a submerged fermentation (SmF) approach, a fungal fermentation-based wheat biorefining technology was developed and is being utilized to produce biofuels, biodegradable polymers, and platform chemicals. The wheat-based biorefining technique has shown complete starch hydrolysis, but protein hydrolysis has not been efficaciously [8]. If starch and protein can be completely hydrolyzed, a variety of media may be created for a variety of fermentations. Aspergillus oryzae microbes are very good at creating proteolytic enzymes, which must be used to thoroughly hydrolyze gluten [9]. Two submerged fermentations for the production of amylolytic and proteolytic enzymes may boost wheat yields [10].

Waste or manufacturing by-product recycling and remanufacturing may improve the efficiency of a product's whole life cycle. This technique is strongly tied to the circular economy, which is becoming an increasingly popular research subject in the UK and throughout the world. In addition to naturally occurring emissions, humans emitted 32 billion metric tonnes (Mt) of carbon dioxide (CO_2) into the atmosphere in 2017. Despite massive research expenditures, the annual worldwide emissions of greenhouse gases (GHGs) have continued to increase inexorably [11].

Agriculture waste may be converted to a variety of valuable products by valorising it, including energy and other usable elements. When it comes to agricultural waste biorefinery demonstrations, performance should be measured by how well

waste is refined from various goods and by products like biofuels and fertilizers to a variety of other products such as heat and power or bioproducts. In biorefining development, cutting-edge technologies such as biotechnology, process chemistry and engineering are all important. A biorefinery is required to maximize the economic value of agricultural waste while simultaneously reducing the quantity of garbage it creates. Wheat straw biomass conversion technologies are attractive to many industries because of the simplicity with which they may be scaled up in the future. Wheat straw biomass conversion has long been the subject of fundamental research. However, only a small portion of this work has been effectively transferred into commercial practise [12].

A thorough knowledge of wheat straw's structure, chemistry, morphology, and how a particular pretreatment and processing alter these properties is required for its use. An accurate database of wheat straw agricultural waste characteristics and composition based on scientific research is essential for any conversion and valorization plan. Since wheat straw has many useful properties and anatomical components, this article will address them for a biorefinery approach that contributes to the success of valorization [13].

In current study, human growth hormone (GH) was found to be superior to other nutrients as a dietary supplement for microbial fermentations in this study. If the A. oryzae SSF solid residues are employed directly as crude enzyme sources, this may result in increased glucose and nutrient concentrations in streams that are already high in glucose and nitrogen. The nutritional composition of the resultant solution was also improved by combining SmF solids with wheat flour hydrolysis. Succinic acid was manufactured from a mixture of GFFH and GH, which provided all of the nutrients necessary for succinic acid synthesis microbiological development.

The paper’s layout for the current study includes the following: 1. introduction; 2. background; 3. review of literature; 4. research methodology; and 5. results and discussion.

2. BACKGROUND

A method in which solid particles with an inter-particle continuous gaseous phase serve either as a substrate or as an inert Solid-state fermentation is the process of growing microbes on inert materials such as rocks or soil without the need of any free water (SSF). As a by-product of the natural decomposition of solid organic matter, people have been employing SsF for millennia in the production of foods and beverages including bread, cheese, tempeh, sake, soy sauce, and even chocolate and coffee. Contrary to popular belief, even though this technique has been used for centuries, it was only recognised as an option for synthesising different compounds in industry in the last few decades [14]. As a result of its usage in cow feed in the 1970s to boost protein content, SSF has seen an increase in research [15].

SSF has long been in use throughout the globe. Mold-ripened cheese is popular in the West, while traditional fermented dishes are popular in the East. Selected microorganisms (s) grow on low moisture-content solid materials in this environment, and it's been recognised as a biotechnology approach and technique with promise. SSF is now a commercially feasible and widely accepted technique for large-scale microbial bioconversion and degradation [16]. As an emerging, interdisciplinary area, sustainable SSF and bioprocess technology development may have applications in the production of food and enzymes as well as chemicals and cosmeceutical/pharmaceutical goods. Recently, academics and businesses from all over the globe have renewed their focus on SSF. This is the situation due to the fact that submerged fermentation (SmF) offers several advantages in the treatment of solid waste. As an alternative to food and feed, SSF has immense potential in the manufacture of high-value, low-volume commodities like enzymes and physiologically active secondary metabolites [17].

Instead of solubilizing nutrients from solid substrates, SSF does away with this step all together. Due to reduced energy needs and reduced waste water, it also reduces the need to monitor and regulate a vast number of factors during fermentation. Since no foam is generated with SSF, it's considerably simpler to recycle the finished product once it's been used. When it comes to raw materials and their capacity to generate different value-added products via microbial bioconversion, SSF offers a lot of versatility. Because of this, contemporary SSF has the greatest potential for producing high-value-added products as a biorefinery target [18].

2.1 Problem with solid-state fermentation: bioreactors are underdeveloped

The use of SSF in industry is still rare, despite its many benefits in terms of both economics and waste management. Nevertheless. In contrast to other fast emerging technologies, the animal feed sector has used SSF the most extensively. Many issues arise with SSF because of the low water content requirements. Because SmF bioreactors consume a lot of water, homogeneous conditions are generated, which facilitates mixing. SmF simulation, bioreactor modelling and design, as well as process control, are all made possible because of this homogeneity. While SSF procedures are more generic, when solid particles, nutrient concentration gradients, a gaseous interphase with volume variations, and microbial growth are included, the system becomes much more complex than it should be.

The sort of substrates used in SSF may also differ from SmF, as opposed to SmF, where water is the most important component. The same technique may be difficult to use when the substrate or microbe is changed, even if it is intended and developed for a particular substrate and set of circumstances. The construction of generic mathematical models to represent processes is complicated by heterogeneity [19]. It's also a significant problem to manage fermentation parameters like moisture content, substrate temperature, or pH during the fermentation process. As a consequence of this [20], large-scale bioreactor design and construction are still pending.

2.2 Potential for circular bioeconomy use of solid-state fermentation

The use of SSF in industry is still rare, despite its many benefits in terms of both economics and waste management. Despite this, in contrast to other rapidly expanding technologies, industrial use of SSF has mostly been restricted to the animal feed industry. Due to its low water content requirements, SSF confronts many difficulties. A further cost-cutting measure is the incorporation of SsF into the integrated production diagram, as seen in Figure 1 [21]. Because water makes up the majority of the working capacity in SmF bioreactors, homogenous conditions are created that make

mixing easier. SmF simulation, bioreactor modelling and design, as well as process control, are all made possible because of this homogeneity. But if you're looking at SSF processes, you'll find that they're far more diversified. When you combine solid particles with concentration gradients of nutrients and microbial development in the interphase, you have one really convoluted system [22].

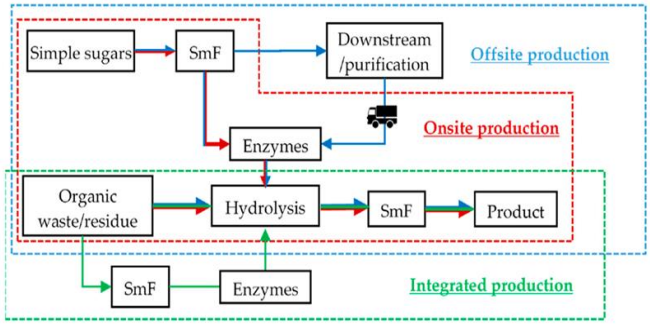

Figure 1. The common offsite production [21]

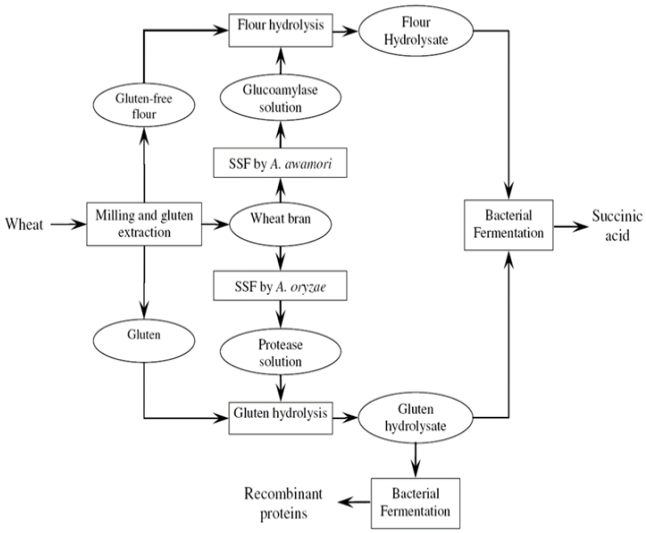

Figure 2. Schematic diagram of the SSF-based biorefining strategy for converting wheat to succinic acid [23]

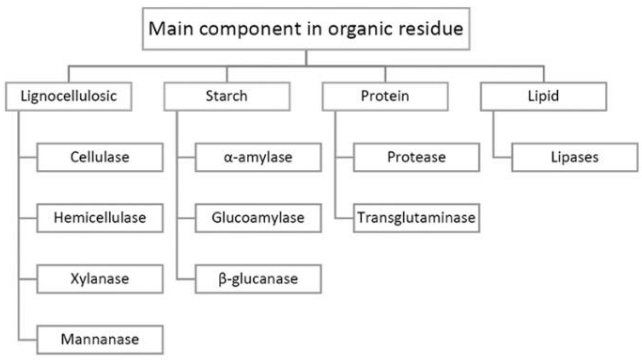

Figure 3. Main enzymes in the decomposition of organic solid residues [21]

The Figure 2 illustrates a unique wheat-based biorefining technique based on wheat bran's SSF. The two SSF performed by Aspergillus awamori and Aspergillus oryzae were combined with flour and gluten hydrolysis to provide generic feedstock media. After that, mixtures of flour hydrolysate and gluten hydrolysate were evaluated for succinic acid fermentation. Any residual gluten hydrolysate might be used to ferment recombinant Escherichia coli cells in order to generate recombinant peptides or proteins. Figure 3 shows some important components in organic residue.

Solid-state fermentation (SSF) has the potential to use much less expensive raw materials, such as wheat bran, than solid-state fermentation (SmF), and therefore enhance process economics. This article describes a new wheat-based biorefining process that utilizes the soluble solid fraction (SSF) of wheat bran. Flour and gluten hydrolysis were used to create general feedstock medium by Aspergillus awamori and A. oryzae, respectively [24]. After that, researchers looked into the possibility of fermentative succinic acid synthesis in a flour hydrolysate/gluten hydrolysate combination. A recombinant E. coli culture may be used to make recombinant peptides and proteins from gluten hydrolysate that has been left behind (unpublished data). For many years, succinic acid has been recognized as a valuable 4-C building block for the synthesis of many high-value derivatives. However, fermentative succinic acid synthesis is still costly when compared to other methods of chemical manufacture [25]. One of the procedures depicted in Figure 4 involved the synthesis of enzymes using wheat bran in fungal SSF. This was then used in hydrolysis reactions to produce nutrient-dense wheat macromolecule hydrolysates (e.g., starch, protein). The nutrient-dense hydrolysate was then fermented to produce the desired products [8].

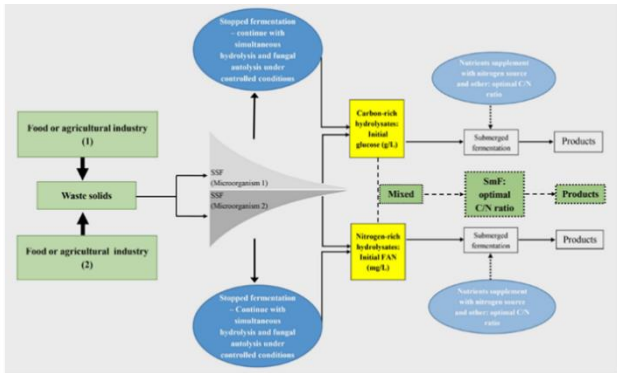

Figure 4. Summary of a novel process strategy for Biorefineries based on SSF to produce generic fermentation feedstock [8]

3. REVIEW OF LITERATURE

Much more research may be done using trash and residue as feedstock, rather than whole wheat. Succinic acid may be made by combining wheat gluten with wheat gluten-free flour. To make glucoamylase and protease extracts, A. awamori and Aspergillus oryzae fermented wheat bran in separate SSF first. Extracts were used to hydrolyze wheat and gluten suspensions. The glucose contents in flour hydrolysate and gluten hydrolysate were 140 g L1 and 3.6 g L1 free amino nitrogen (FAN), respectively.

The glucose and nitrogen-enriched streams were then combined and utilized to produce succinic acid at a concentration of 22 g L1 (and 64 g L1 with $MgCO_3$ added to the medium). The authors hypothesized that, in addition to supplying Mg for development, adding $MgCO_3$ increases the

availability of CO_2 in the broth, explaining the improvement. For this reason, instead of extracting first, they proposed that adding the solid SSF residue to the wheat and gluten suspension is a preferable method.

More experiments will employ trash and residues as fuel, rather than whole wheat, as the starting point. What happens when you mix wheat gluten with gluten-free flour to generate succinic acid? Wheat bran was fermented first by A. awamori and Aspergillus oryzae in separate SSF, yielding glucoamylase and protease extracts, respectively. The extracts were used to hydrolyze the wheat and gluten suspensions before they were added back into the mixture. A total of 140 g L1 of glucose was found in flour hydrolysate, whereas the total free amino nitrogen (FAN) concentration was 3.6 g L1.

The results indicate once again how valuable the SSF step is since it not only delivers enough enzymes during hydrolysis to liberate free sugars and FAN, but it also does so at acceptable concentrations. There are a variety of alternatives to succinic acid, as noted by the authors, including a broad range of bakery waste feedstock [4].

The benefits of sequentially using SSF and SmF were also shown. Enzyme synthesis in SSF was fuelled by bakery waste (pastries). Hydrolysis was performed on the resulting crude enzyme source. The authors, unlike previous investigations, used kitchen wastes for hydrolysis and subsequent SmF to create succinic acid [5].

In all, 31.9 g L1 of glucose and 280 mg L1 of FAN were recovered at the conclusion of the hydrolysis. When commercial enzymes were used, a glucose concentration of 143 g L1 was achieved; this result was lower. There are also some drawbacks to using so much sugar, such that it inhibits the development of certain bacteria, including the pathogen Actinobacillus succinogene, when glucose concentrations are over 70 g L1 [6].

It's got a few standout characteristics. Prior to examining wheat milling residues and bakery wastes as feedstocks for hydrolysis and SmF, the authors turned to kitchen trash as a starting point. This followed the general trend of using starch instead of traditional feedstocks (simple sugars) at first. Kitchen trash is much more diversified when compared to other types of garbage, such as starch, and it is also produced in vast numbers [7].

There are numerous advantages from a practical standpoint to utilizing different substrates for the SSF (bakery wastes), hydrolysis, and SmF (kitchen wastes). To manufacture enzymes, it is logical to assume that certain wastes would function better than others.

The flexibility gained by mixing diverse substrates at various phases of the process, such as enzyme activity, sugar concentrations and FAN in hydrolysis, rises as a result, and more choices are provided in terms of achieving particular goals. Furthermore, SSF provides an aimed solution for bioconversion in this particular instance when the excessive concentration of sugars (obtained by commercial enzymes) is harmful [9].

The synthesis of lactic acid from food wastes uses a similar method. Amylases and proteases were produced by A. awamori and A. oryzae, respectively, using SSF of kitchen trash. As a further step, 1 L of deionized water was used to suspend 14 g of SSF residues from both strains together with 300 g of fresh kitchen trash for hydrolysis. Highly elevated glucose and FAN values were found [10]: 1000.2 mg L1 for glucose and 1081 mg L1 for FAN.

The hydrolysates have been utilized in SmF to produce 94 g L1 of lactic acid. A techno-economic evaluation of the process simulation in an industrial environment was also performed by the authors and included the SSF step for the synthesis of enzymes on-site [11].

Another study employed SSF to make a nutrient-rich supplement instead of the expensive yeast extract and inorganic chemicals that are often used in fermentations. Sunflower meal, a protein-rich byproduct of sunflower oil manufacturing, was used as a substrate in SSF. Other nutrients were not supplied to the mushroom A. oryzae, which was grown only on the meal (5 g). To learn more about sunflower meal's hydrolysis capabilities, hydrolysis tests were performed using fermented SSF leftovers.

FAN is released from proteins by proteolytic enzymes, and higher temperatures, such as those used in hydrolysis, enhance fungal autolysis, which releases nutrients like those found in yeast extract. Cupriavidus necator produced PHA using the hydrolysate as a nitrogen source in a SmF containing glycerol (a carbon source).

This has happened before, as well, with the ratio of fermented solids to fresh solids for the hydrolysis step dropping from about 1:9 to as low as 1:18. The best results for FAN were obtained by hydrolyzing with a total solid (dry basis) concentration of 90 g L1 (5 g of which was made up of SSF residues) [14].

Because the amount of SSF solids required is so small, SSF experiments can be carried out on a small scale. This is important. In a related work, rapeseed meal leftovers were used in SSF to provide a nitrogen-rich medium. Using a 1:10 combination of fermented meal and fresh meal, SSF was followed by liquid hydrolysis. Unrefined glycerol and nitrogen-rich substances were combined to create PHA [15].

SSF was considered as a precursor as an alternative. As a substrate support and enzyme inducer, sugarcane bagasse soaked with a nutrient solution was employed in their studies to produce A. Niger.

To the leftover fermented particles, a liquid nutrient solution was added, and a SmF was performed to generate cellulases. Afterwards, they looked at the difference in cellulose efficiency between their sequential technique and a single-step flash method. In comparison to SmF alone, a pre-SSF step had a 3-fold increase in endoglucanase production, indicating a favourable impact [17].

According to the scientists, sugarcane bagasse has an enzyme inducer effect and the shape of the fungus changes from pellets to scattered filamentous when grown on a solid-state pre-culture. There is yet another example of how to utilize anaerobic SsF as a pretreatment step with microalgae such as Chlorella sp. TISTR 8411 with SsF. Hydrolysis was performed on the leftovers, followed by dark fermentation to produce biohydrogen [18].

4. RESEARCH METHODOLOGY

4.1 Microorganisms

In SSF, amylolytic and proteolytic enzymes were produced by A. awamori and A. oryzae, respectively. Previous research has detailed how to store and sporulate them for use in inoculum production [8, 17, 18,]. For succinic acid fermentations, scientists used Actinobacillus succinogenes ATCC 55618.

4.2 Raw materials

Consort, a soft wheat type, was utilized in this investigation. An earlier study detailed the wheat's chemical makeup. To make wheat flour and bran, the grain was ground in a Bühler laboratory mill. The traditional Martin procedure was used to get rid of the gluten. This recipe calls for approximately 1 litre of water for 200 grammes of wheat flour (dry basis).

4.3 Solid-state fermentation

The current study used two Duran bottles filled with wheat bran and autoclaved them for 30 minutes at 121°C by A. oryzae and A. awamori. Following addition of sterile water to raise the moisture level of the bran to 60%, the bottles were individually infected with A. awamori and A. oryzae to obtain the desired inoculum sizes (4 106 and 1 106 spores per g wheat bran for A. awamori and A. oryzae). Six 14 cm Petri plates were filled with about 15 g of wheat bran and incubated at 30°C for 96 hours with A. awamori or 48 hours with A. oryzae, depending on the strain.

4.4 Enzyme extraction

Each Petri dish was filled with 1 g of fermented mash and 10 ml of distilled water in a 1:10 ratio. Enzyme extraction was combined in a 1-liter Duran bottle for an hour at room temperature using a magnetic stirrer. Wheat and gluten hydrolysis was carried out using an enzyme solution filtered through Whatman No. 1 filter paper.

4.5 Flour and gluten hydrolysis

Nitrogen was generated using the protease-rich solution, whereas glucose was hydrolyzed to yield a glucose-rich stream (designated as Gluten Hydrolysate, GH). A gluten-free flour suspension comprising around 160 g of starch was gelatinized for 20 minutes at 70°C in a 2-liter glass jacketed reactor. The agitation was performed at a speed of 500 revolutions per minute. It was necessary to decrease the temperature of the reactor to 55°C before adding different quantities of glucoamylase solution (0.1-0.6 l). Before being chopped into bits by passing it through a 500-lm filter, the gluten that was extracted using the Martin method was autoclaved at 121°C for 15 minutes. In a 2-liter glass jacketed reactor, the pieces were agitated at 500 revolutions per minute with the protease solution. Throughout the experiment, the temperatures were kept at 55 C for the flour and gluten hydrolyses, respectively. In order to initiate hydrolysis with a pH of 4.5, 2M H_2SO_4 was added to both enzyme solutions first. In the experiment, they were not controlled since they stayed at or near this value throughout the whole time period. A 24-hour turnaround was required for the response. Hydrolysis and solid-state fermentation in Petri plates were done three times each [24].

4.6 Succinic acid fermentation

The inoculation technique and batch fermentation conditions for succinic acid fermentation were previously described in detail in another study. GFFH and GH were mixed to get the desired glucose and free amino nitrogen (FAN) concentrations. The mixture was sterilized in an aseptic manner in a 0.2 lm membrane filter before being supplied to the bioreactor. Succinic acid production was investigated to see how $MgCO_3$ affected it by introducing different amounts of it (2, 30 and 50 g l 1). The fermentation's pH was kept between 6.6 and 6.8 the whole time thanks to a 10 M NaOH solution. To compare, NaOH consumption varied from 20–100 ml with and without $MgCO_3$, as well as dependent on the starting concentrations of $MgCO_3$ and glucose. Batch fermentation results were replicated and only varied by 10%.

4.7 Analytical techniques

As previously mentioned, the OD, glucose, and fermentation metabolites were all measured. The activity of glucoamylase was measured in the manner indicated. The quantity of glucoamylase needed to produce one milligramme of glucose per minute under the test conditions was one unit (U). As previously stated, the activity of the protease enzyme was assessed. Under the test conditions, the quantity of protease needed to release one milligramme of FAN per minute was defined as one unit. It was found that the mineral composition of the soil was composed of phosphorus, potassium, magnesium, sodium, and total Kjeldahl nitrogen (TKN). Using a GC/MS analysis kit (EZ: -fast GC/MS protein hydrolysate kit, Phenomenex, UK), the free amino acid concentrations were measured, and the method was previously reported [25].

5. RESULTS AND DISCUSSION

This study synthesized glucoamylase (using A. awamori) and protease from wheat bran utilizing just a Bühler mill's SSF as a substrate (using A. oryzae).

5.1 Production of glucoamylase and protease

Wheat straw has been generally utilized in strong state maturations for the development of cellulase and an expansive scope of dampness substance from half to 86% has been investigated. The current study examined a somewhat high dampness range to speed up the development of A. Niger and cellulase creation.

Table 1. The impact of moisture content on cellulase production

Sr. No	Liquid to solid ratio (w/w)	Filter paper activity (U/g)
1	5.1	4.10
2	6.1	5.0
3	7.1	4.9
4	7.5.1	5.5
5	8.1	4.0
6	9.1	3.10

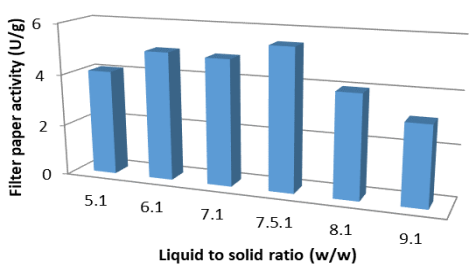

Figure 5. Moisture content on cellulase production

Table 1 shows that the impact of moisture content on cellulase production. Liquid to solid ratio for 5.1 is 4.10 for Filter paper activity (U/g), same for 6.1 is 5.0, for 7.1 is 4.9, for 7.5.1 is 5.5, for 8.1 is 4.0, for 9.1 is 3.10 respectively. Figure 5 shows moisture content on cellulase production.

5.2 Flour and gluten hydrolysis

In the solid-state maturations, the wheat straw was autoclaved before the vaccination. The autoclave sanitised the substrate and additionally changed the wheat straw morphology, which could be considered a gentle aqueous pre-treatment of the wheat straw. In this review, two other wheat straw alteration strategies, weaken corrosive and corrosive splashing, were explored with the plan to work on parasitic development and cellulase creation. These outcomes were then contrasted with maturations utilising autoclaved wheat straw and rough (non-adjusted) wheat straw. Table 2 shows the effects of different modification methods on cellulase production. For Day 1 Non-treated ratios shows that 4.37, for Day 3 it shows 5.83 and for Day 5 it shows that 3.89 respectively.

Aqueous extraction of SSF solids generated glucoamylase and protease-rich solutions that were used in the breakdown of wheat and gluten to create glucose-and nitrogen-rich streams, respectively. GFFS and wheat gluten were the substrates utilized in the flour hydrolysis. You may see how much glucose is produced by adding different concentrations of glucoamylase-rich solution (0.1–0.6 v/v) to GFFS in various ratios (see graph). In all ratios, the GFFH had a glucose concentration more than 110 g l 1. To make succinic acid commercially, you'll need a fermentation medium with a glucose concentration of at least 100 g l 1.

With varying gluten loadings (4-110 g), a protease-rich solution was used to hydrolyze the gluten. It was discovered that adding 65 grammes of dried gluten to one litre of a protease-rich solution produced a nitrogen-rich stream consisting of 3.6 grammes of 1 one FAN. The FAN concentration generated by an A. awamori SmF on whole wheat flour was found to be 2.3 times larger than that produced by fungal autolysis using residual solids from the A. awamori SmF. There were differences found when looking at how many amino acids were present in the GH compared to those found in yeast extract and fungal autolysate [26]. Figure 6 shows Effect of substrate to enzyme ratio on glcose production from glten free flour.

For fermentative production of succinic acid, it's plausible to assume that GFFH and GH are comparable enough to be alternate wheat-derived media. GFFH and GH were the sole media utilized in the fermentations. Starting values were 50 g l^{-1} glucose and 1000 mg l^{-1} FAN. This yield of succinic acid was produced by incubating 1 glucose for 28 hours, which resulted in 23.5% succinic acid production. A study using SSF-based raw materials demonstrated that the nutrients required to ferment succinic acid were present. GFFH and GH fermentations produced less succinic acid than the semi-defined medium fermentations [27]. The absence of protection might have resulted in this.

As a result, it was hypothesized that adding magnesium might enhance the synthesis of succinic acid. Succinic acid concentrations are measured in fermentations with $MgCO_3$ values of 2, 30, and 50 g l 1. When beginning with merely 2 g l 1 $MgCO_3$, researchers were able to produce succinic acid concentrations of 31.8 g l 1 in GFFH and GH fermentation. GFFH, GH, and a starting $MgCO_3$ concentration of 50 g/l generated the greatest succinic acid throughout fermentation (37.7 g l 1). When $MgCO_3$ is used instead of the control fermentation, the yield increases by 61 percent [28]. Another advantage was obtained by mixing 30 grammes of $MgCO_3$ with 100 grammes of glucose in the wheat-derived feedstock. 64.2 grammes of l^{-1} succinic acid were produced as a result. This is the most succinic acid that has ever been produced in a natural medium [29-31]. Figures 7 and 8 display Glucose and fermentation product profiles for a succinic acid fermentation carried out with GFFH, GH and 30 gl^{-1} $MgCO_3$.

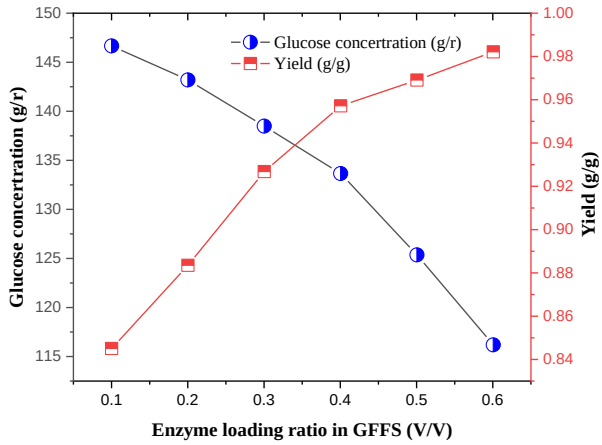

Figure 6. Effect of substrate to enzyme ratio on glcose production from glten free flour (The glucoamylase activity was 4.4 U ml^{-1})

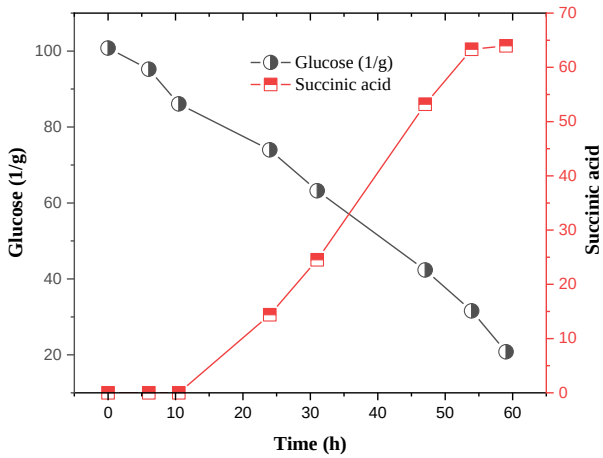

Figure 7. Glucose product profile

Table 2. Effect of different modification methods on cellulase production

Time (day)	Non-treated	Autoclave	Diluted acid	Acid soaking
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	4.37 ± 1.37	4.33 ± 0.38	8.65 ± 0.58	7.51 ± 1.20
3	5.83 ± 0.68	9.51 ± 1.64	10.2 ± 0.13	5.11 ± 1.08
5	3.89 ± 0.96	5.55 ± 0.54	4.43 ± 0.07	4.73 ± 0.93

Table 3. Comparison of succinic acid fermentations using various media

Sr. No	Fermentation media	Succinic acid concentration (g l^{-1})	Succinic acid productivity (g l^{-1} h^{-1})	Yield [d] (g g^{-1})
1	**Semi-Defined Medium**	27.9	20.56	16.62
2	**FH and FA[a]**	15.9	10.31	5.47
3	**GFFH[b] and GH**	23.5	12.97	9.60
4	**GFFH[b], GH and 2 g l^{-1}**	31.8	16.30	13.66

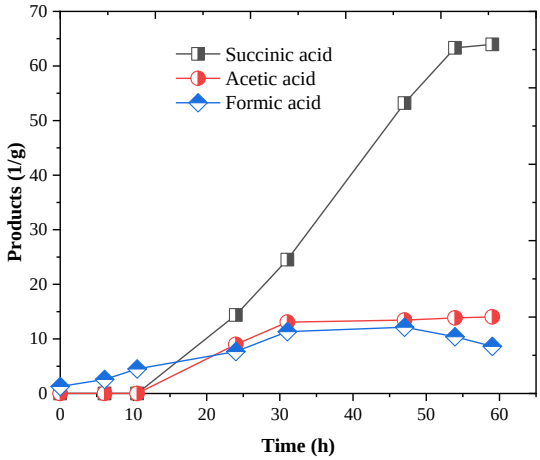

Figure 8. Fermentation product profiles for a succinic acid fermentation carried out with GFFH, GH and 30 gl^{-1} $MgCO_3$

Table 3 shows the comparison of succinic acid fermentations using various media (Semi-Defined Medium). Succinic acid concentration for Semi-Defined Medium is 27.9 (g l1), Succinic acid productivity for Semi-Defined Medium is 20.56 (g l1 h1), and Semi-Defined Medium yield is 16.62 (g g1).

Also, the table shows the comparison of succinic acid fermentations using various media (FH and FAa). Succinic acid concentration for FH and FAa is 15.9 (g l1), Succinic acid productivity for FH and FAa is 10.31 (g l1 h1), and yield for FH and FAa is 5.47 g l1.

The comparison of succinic acid fermentations using various media (GFFHb and GH). The Succinic acid concentration for GFFHb and GH is shown to be 23.5 (g l^{-1}). The Succinic acid productivity for GFFHb and GH is shown to be 12.97 (g l^{-1} h^{-1}) and the yield for GFFHb and GH is shown to be 9.60 (g g^{-1}).

Furthermore, comparison of succinic acid fermentations using various media (GFFHb, GH, and 2 g l^{-1}). Succinic acid concentration for GFFHb, GH, and 2 g l^{-1} is 31.8 (g l^{-1}), Succinic acid productivity for GFFHb, GH, and 2 g l^{-1} is 16.30 (g l^{-1} h^{-1}), and Succinic acid yield for GFFHb, GH, and 2 g l^{-1} is 13.66 (g g^{-1}).

6. CONCLUSIONS

SSF may utilize wheat bran as a low-cost enzyme substrate. Glucoamylase and protease were produced using wheat bran in this research through SSF. Near-optimal conditions resulted in about 48 and 64 U g 1 of each enzyme, respectively. Other studies have also discovered similar glucoamylase and protease activity in SSF. For the creation of fermentation medium from wheat, the SSF-based approach produced comparable glucoamylase activity but almost three times more protease activity than the SmF-based strategy. It's possible to use gluten hydrolysis as a nitrogen-rich stream for various microbial ferments because of the high protease activity. Future biorefineries will use all biomass components, resulting in little waste. This wheat-based biorefinery's production method utilizes all of the grain's components (bran, starch, and gluten). With the new approach, amylolytic and proteolytic solutions derived from wheat bran SSF are used to hydrolyze wheat starch and protein, producing glucose and nitrogen-rich streams.

There will be amylolytic-rich solutions made. Using a glucose-rich stream as an example, one may attain glucose concentrations of 140 g l 1 and glucose conversion yields of 0.93 (GFFH). This glucose concentration is completely out of wack when it comes to commercial succinic acid fermentation (about 100 g l 1). This gluten hydrolysis solution, developed by A. oryzae SSF, provided a nitrogen-rich stream with up to 3.6% more free amino acids per litre than the SmF-based technique. According to the results, the GH contained higher concentrations of all detected amino acids than the FA or a solution containing 10 g l 1 yeast extract. This study revealed that human growth hormone (GH) was superior to other nutrients as a dietary supplement for microbial fermentations. This might perhaps lead to increased concentrations of glucose and nutrients in streams that are already rich in glucose and nitrogen if the A. oryzae SSF solid residues are used directly as crude enzyme sources. The enzymes would be introduced directly to the GFFS or gluten suspension, rather than being transferred to aqueous solutions. When SSF masses are extracted by floating them in water and trying to extract the enzymes and nutrients, enzyme and nutrient loss may occur. According to research, A. awamori does not grow or absorb nutrients at 55 ℃ in the absence of oxygen. The nutritional makeup of the resulting solution was also enhanced by using SmF solids in conjunction with wheat flour hydrolysis. This potential will be further explored in the future, according to the plan. Succinic acid was synthesized from a mixture of GFFH and GH, which provided all of the nutrients required for microbial growth for succinic acid production. According to the study, fructose fermentations generated 1.5 and 3.1 times more succinic acid than fructose fermentations utilizing just FH and FA. Most likely, this was due to a higher concentration of amino acids in the GH than in the placebo.

Glutamate and methionine are only two examples of the essential amino acids needed for succinic acid production. In comparison to FA, methionine and glutamate levels in GH were 5.7 and 3.2 times higher, respectively. Methionine or glutamate-free medium failed to support microbial growth or succinic acid production (Data not shown). In comparison to semi-defined media, wheat-derived media contain lower mineral contents, which may limit succinic acid biosynthesis. It was shown that adding $MgCO_3$ boosted succinic acid concentration by 36%, as well as productivity in both GFFH and GH when compared to the control fermentation. Succinic acid fermentations using natural-derived medium have produced the highest succinic acid concentrations ever reported, with a starting $MgCO_3$ concentration of 30 g l 1. Additionally, Mgprovision and CO_2 are released when magnesium carbonate reacts with fermentable organic acids. CO_2 is more quickly absorbed because it is more readily

accessible in the A. succinogenes cells' microenvironment. CO_2 has a crucial role to perform in guiding metabolic flux to the phosphoenolpyruvate node.

REFERENCES

[1] López-Gómez, J.P., Manan, M.A., Webb, C. (2020). Solid-state fermentation of food industry wastes. In Food Industry Wastes, pp. 135-161. https://doi.org/10.1016/B978-0-12-817121-9.00007-3

[2] Pandey, A. (1992). Recent process developments in solid-state fermentation. Process Biochemistry, 27(2): 109-117. https://doi.org/10.1016/0032-9592(92)80017-W

[3] Pandey, A., Soccol, C.R., Larroche, C. (2008). Current Developments in Solid-State Fermentation. Springer Science & Business Media.

[4] López-Gómez, J.P., Venus, J. (2021). Potential role of sequential solid-state and submerged-liquid fermentations in a circular bioeconomy. Fermentation, 7(2): 76. https://doi.org/10.3390/fermentation7020076

[5] Soccol, C.R., da Costa, E.S.F., Letti, L.A.J., Karp, S.G., Woiciechowski, A.L., de Souza Vandenberghe, L.P. (2017). Recent developments and innovations in solid state fermentation. Biotechnology Research and Innovation, 1(1): 52-71. https://doi.org/10.1016/j.biori.2017.01.002

[6] Shinkawa, S., Mitsuzawa, S. (2020). Feasibility study of on-site solid-state enzyme production by Aspergillus oryzae. Biotechnology for Biofuels, 13(1): 1-15. https://doi.org/10.1186/s13068-020-1669-3

[7] Costa, J.A., Treichel, H., Kumar, V., Pandey, A. (2018). Advances in solid-state fermentation. In Current Developments in Biotechnology and Bioengineering, pp. 1-17. https://doi.org/10.1016/B978-0-444-63990-5.00001-3

[8] Abdul Manan, M., Webb, C. (2017). Modern microbial solid state fermentation technology for future biorefineries for the production of added-value products. Biofuel Research Journal, 4(4): 730-740. https://dx.doi.org/10.18331/BRJ2017.4.4.5

[9] Thomas, L., Larroche, C., Pandey, A. (2013). Current developments in solid-state fermentation. Biochemical Engineering Journal, 81: 146-161. https://doi.org/10.1016/j.bej.2013.10.013

[10] Oviedo-Lopera, J.C., Zartha-Sossa, J.W., Zapata-Ruiz, D.L., Bohorquez-Naranjo, I., Morales-Arevalo, K.S. (2020). Systematic review and study of s curves for biomass quantification in solid-state fermentation (SSF) and digital image processing (DIP) applied to biomass measurement in food processes. Recent Patents on Biotechnology, 14(3): 194-202. https://doi.org/10.2174/1872208314666200312094447

[11] Mitchell, D.A., Pitol, L.O., Biz, A., Finkler, A.T.J., de Lima Luz, L.F., Krieger, N. (2019). Design and operation of a pilot-scale packed-bed bioreactor for the production of enzymes by solid-state fermentation. Solid State Fermentation, 27-50. https://doi.org/10.1007/10_2019_90

[12] Arora, S., Rani, R., Ghosh, S. (2018). Bioreactors in solid state fermentation technology: Design, applications and engineering aspects. Journal of Biotechnology, 269: 16-34. https://doi.org/10.1016/j.jbiotec.2018.01.010

[13] Manan, M.A., Webb, C. (2017). Design aspects of solid state fermentation as applied to microbial bioprocessing. Journal of Applied Biotechnology & Bioengineering, 4(1): 1-25. http://http//dx.doi.org/10.15406/jabb.2017.04.00094

[14] Ashok, A., Doriya, K., Rao, D.R.M., Kumar, D.S. (2017). Design of solid state bioreactor for industrial applications: an overview to conventional bioreactors. Biocatalysis and Agricultural Biotechnology, 9: 11-18. https://doi.org/10.1016/j.bcab.2016.10.014

[15] Leite, P., Sousa, D., Fernandes, H., Ferreira, M., Costa, A.R., Filipe, D., Gonçalves, M., Peres, H., Belo, I., Salgado, J.M. (2021). Recent advances in production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes. Current Opinion in Green and Sustainable Chemistry, 27: 100407. https://doi.org/10.1016/j.cogsc.2020.100407

[16] Kumar, V., Ahluwalia, V., Saran, S., Kumar, J., Patel, A. K., Singhania, R.R. (2020). Recent developments on solid-state fermentation for production of microbial secondary metabolites, challenges and solutions. Bioresource Technology, 323: 124566. https://doi.org/10.1016/j.biortech.2020.124566

[17] Verduzco-Oliva, R., Gutierrez-Uribe, J.A. (2020). Beyond enzyme production: solid state fermentation (SSF) as an alternative approach to produce antioxidant polysaccharides. Sustainability, 12(2): 495. https://doi.org/10.3390/su12020495

[18] Soccol, C.R., Vandenberghe, L.P., Rodrigues, C., Medeiros, A.B.P., Larroche, C., Pandey, A. (2008). Production of organic acids by solid-state fermentation. In Current Developments in Solid-State Fermentation, pp. 205-229. https://doi.org/10.1007/978-0-387-75213-6_10

[19] Cerda, A., Artola, A., Barrena, R., Font, X., Gea, T., Sánchez, A. (2019). Innovative production of bioproducts from organic waste through solid-state fermentation. Frontiers in Sustainable Food Systems, 3: 63. https://doi.org/10.3389/fsufs.2019.00063

[20] Sadh, P.K., Duhan, S., Duhan, J.S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: A review. Bioresources and Bioprocessing, 5(1): 1-15. https://doi.org/10.1186/s40643-017-0187-z

[21] Johnson, E. (2016). Integrated enzyme production lowers the cost of cellulosic ethanol. Biofuels, Bioproducts and Biorefining, 10(2): 164-174. https://doi.org/10.1002/bbb.1634

[22] Lizardi-Jiménez, M.A., Hernández-Martínez, R. (2017). Solid state fermentation (SSF): Diversity of applications to valorize waste and biomass. 3 Biotech, 7(1): 44. https://doi.org/10.1007/s13205-017-0692-y

[23] Du, C., Lin, S.K.C., Koutinas, A., Wang, R., Dorado, P., Webb, C. (2008). A wheat biorefining strategy based on solid-state fermentation for fermentative production of succinic acid. Bioresource Technology, 99(17): 8310-8315. https://doi.org/10.1016/j.biortech.2008.03.019

[24] Abu Yazid, N., Barrena, R., Komilis, D., Sánchez, A. (2017). Solid-state fermentation as a novel paradigm for organic waste valorization: A review. Sustainability, 9(2): 224. https://doi.org/10.3390/su9020224

[25] Kosseva, M.R. (2013). Food Industry Wastes: Chapter 5. Recovery of Commodities from Food Wastes Using Solid-State Fermentation. Elsevier Inc. Chapters.

[26] Couto, S.R., Sanromán, M. A. (2006). Application of solid-state fermentation to food industry-A review.

Journal of Food Engineering, 76(3): 291-302. https://doi.org/10.1016/j.jfoodeng.2005.05.022
[27] Raimbault, M. (1998). General and microbiological aspects of solid substrate fermentation. Electronic Journal of Biotechnology, 1(3): 26-27. http://dx.doi.org/10.4067/S0717-34581998000300007
[28] Pandey, A., Soccol, C.R., Mitchell, D. (2000). New developments in solid state fermentation: I-bioprocesses and products. Process Biochemistry, 35(10): 1153-1169. https://doi.org/10.1016/S0032-9592(00)00152-7
[29] Pandey, A. (2003). Solid-state fermentation. Biochemical Engineering Journal, 13(2-3): 81-84. https://doi.org/10.1016/S1369-703X(02)00121-3
[30] Castilho, L.R., Mitchell, D.A., Freire, D.M. (2009). Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation. Bioresource Technology, 100(23): 5996-6009. https://doi.org/10.1016/j.biortech.2009.03.088
[31] Viniegra-González, G., Favela-Torres, E. (2006). Why solid-state fermentation seems to be resistant to catabolite repression? Food Technology and Biotechnology, 44(3): 397-406. https://hrcak.srce.hr/109941.