Effect of low-cost substrate on the fatty acid profiles of Mortierella alpina CBS 754.68 and Wickerhamomyces siamensis SAKSG

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ABSTRACT
Fungi and yeast species as good sources of arachidonic acid and polyunsaturated fatty acids have been attracting a lot of attention from scientists in recent years. In this study, we used the oleaginous yeast Wickerhamomyces siamensis SAKSG isolated from the gills of trout caught from the Caspian Sea. Low-cost polysaccharides hydrolyzed by enzymatic digestion of immobilized whole cells of Bacillus amyloliquefaciens ATCC 23350 served as substrates to evaluate the effects of each on the biomass and the fatty acid profiles of Mortierella alpina CBS 754.68 and Wickerhamomyces siamensis SAKSG. By using sprouted wheat, millet and wheat as starch sources adjusted to 150 g/L, we obtained reducing sugar content of 102.57, 57.50 and 51.62 g/L, respectively. The highest and lowest dry weight (biomass) of M. alpina was obtained in sprouted wheat (3.7% of media) and rice-formulated media (1.72% of media), respectively. Each formulated media significantly affected the profiles of M. alpina fatty acids. The highest content of linoleic acid (41.873%), oleic acid (30.061%) and arachidonic acid (27.054%) was obtained with corn, rice and potatoes used as the carbon sources. The yeast lipid content was high, consisting of C6 up to C12 fatty acids, accounting for about 55.7% of the total fatty acids. Some amount of docosahexaenoic acid (DHA) and (ecosapentanoic acid) EPA was also observed in W. siamensis.

Introduction
Arachidonic acid (5.8.11.14 eicosatetraenoic acid) as a structural fatty acid plays important roles in human health. This essential fatty acid contributes to neural network and development of preterm infants; therefore, many organizations, such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), have recommended using it in infant milk formulas [1,2]. The scarcity of arachidonic acid in animal and plant tissues as well as the environmental pollutants of marine oils, which are the main sources of arachidonic acid, have motivated the search for reliable, safe sources of this fatty acid [3,4]. Some good sources of polyunsaturated fatty acids (PUFAs) for industrial purposes are fungi and yeasts due to their rapid growth rate, high tolerance to a wide pH range and ability to grow in minimal media [5]. For years, many researchers have attempted to produce high amounts of long-chain unsaturated fatty acids from Mortierella alpina species using industrial oleaginous fungi [6]. Arachidonic acid oil produced by fermentation of Mortierella alpina has been used as a supplement in infants’ dry milk formula around the world [7]. Notably, the culture medium highly influenced the microorganism growth as well as the lipid production. That is why research has focused on optimisation of the substrates to achieve a commercial fermentation product [1,8–10]. The cost of the raw materials, especially the carbon sources, significantly affects the final price of the fermentation products, so using agricultural, industrial and factory waste was considered to be a critical factor in reducing the final cost of fermentation products. To date, many agricultural-industrial wastes are applied as low-cost substrates to produce microbial oil [11]. Some well-known species of oleaginous fatty acids, such as Mortierella alpina, have been widely employed both in research and for industrial goals. On the other hand, new oleaginous species that are capable of producing PUFAs and lipids could be considered as an effective step to industrialise a wider range of microorganisms for commercial purposes. Therefore, in the present study, we used α-amylase produced from immobilized whole cells of Bacillus amyloliquefaciens ATCC 23350 to hydrolyze low-cost starch sources including sprouted wheat, wheat, millet, oat, potato, corn, rice and Acanthophyllum bracteatum. The substrates were used to produce...
biomass and unsaturated fatty acids from *M. alpina* and a new isolated oleaginous marine yeast *Wickerhamomyces siamensis* SAKSG.

**Materials and methods**

**Culture conditions**

*Bacillus amyloliquefaciens* ATCC 23350 was inoculated in nutrient broth medium at 27 °C and 150 rpm for 18 h [12]. Mycelia suspension of *Mortierella alpina* CBS754.68 was applied for inoculation in the fermentation medium [13]. The seed culture used for isolating yeasts contained glucose monohydrate (30 g/L) and yeast extract (10 g/L). The isolated yeasts were grown at 26 °C and 170 rpm for 72 h, and 3%-10% of seed cultures were added to the fermentation medium. The compounds ((NH₄)₂SO₄ (2 g/L), KH₂PO₄ (3 g/L) and MgSO₄ (1.5 g/L) were also used as mineral elements in the fermentation media [6].

*B. amyloliquefaciens* ATCC 23350 immobilization

During this step, the *B. amyloliquefaciens* ATCC 23350 cells were centrifuged at 10 000 × g at 4 °C for 10 min [14]. Then, the cell sediment was washed three times in distilled water. Finally, it was added to the initial solution of gel formation for bead immobilization [15].

**Isolation of oleaginous yeast**

In the present study, four fish species, including common carp, mullet, zander and trout, were chosen. The surface of the fish body was washed with alcohol 70%. Then, 1 g of fish gills was removed with a sterile knife. The samples were incubated at 25 °C in yeast extract glucose chloramphenicol agar (YGC agar). A single yeast colony was isolated and, after growth in PDA, was maintained in the refrigerator at 4 °C [16].

**Species identification**

To identify the yeast species which produces the highest amount of lipids, the yeast sample was first incubated in a seed culture medium for 48 h; then, DNA was extracted using a DNA extraction kit (K721, Thermo, USA). The D1/D2 26S rDNA was amplified by forward (5’-GCATATCATAACGGCAGG AAAAG –3) and reverse primer (5’GGTCCGTGTTCATAGACGG-3’) by polymerase chain reaction (PCR) [17]. The PCR product (680-bp long) was extracted from the gel and sent to the TakapuZist Company to determine the nucleotide sequence. After sequence determination, they were blasted using the NCBI website, and Mega5 software was used to draw a phylogenetic tree.

**Starch extraction**

To extract starch, potatoes, *Acanthophyllum bracteatum*, sprouted wheat, wheat, millet, oat, rice and corn were immersed in boiling distilled water (500 mL), crushed and cooked at 100 °C for 7 min. The starch content was measured by the method of Thivend et al. [18] and was adjusted to 150 g/L.

**Production and extraction of alpha-amylase**

After adding 300 beads of immobilized whole cells to the production medium, the samples were incubated at 37 °C and 150 rpm for 18 h. When the fermentation process was completed, the medium was centrifuged at 10 000 × g at 4 °C for 10 min. Finally, the supernatant was applied as an enzyme source [14]. The enzyme solution was added to each medium (pH 5.9) containing 150 g/L starch from wheat, oat, potato, rice, corn, millet or sprouted wheat, and all were kept at 65 °C for 3 h and 15 min for starch hydrolysis.

**Fermentation medium**

Based on previous studies, to produce the highest amount of unsaturated fatty acids from *M. alpina*, reducing sugar (40 g/L) as the carbon source and soybean powder (20 g/L), with peptone (2.5 g/L) as the nitrogen source; KH₂PO₄ (3 g/L), MgSO₄·7H₂O (0.2 g/L), KNO₃ (2.5 g/L) as minerals were used. The pH was adjusted at 6.0, and the fungal mycelia suspension was used for fermentation (at 21 °C temperature and 180 rpm) for 10 days. The fermentation for yeast was conducted at 28 °C and 180 rpm for 5 days with under the same culture conditions.

**Analytical methods**

The dry weight of the biomass and the content of reduced sugar were determined as previously described [6,19]. To determine and measure fatty acids, the lipids must first be decomposed into fatty acids and glycerol. The derivation of fatty acids was done by the method of Metcalfe et al. [20]. Subsequently, fatty acids were analysed by gas chromatography (Unicam 4600, England) with flame ionization detection (FID) [21].

**Data analysis**

Data are presented as mean values with standard deviation (±SD) from triplicate experiments. Data analysis was...
performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software.

Results and discussion

Number of immobilized B. amyloliquefaciens cells

After 18 h cultivation in the nutrient broth, the number of microorganisms in 100 mL of the medium was $10^{15}$ cfu/mL. As there were 1500 beads in 110 mL of the solution (7 mL of suspension of the bacteria + 3 mL of distilled water + 100 mL of beads solution), there were $3.33 \times 10^{12}$ bacteria in every bead.

Effect of B. amyloliquefaciens extracellular enzymes in media with various sources of starch

During this step, the pH value of the starch substrates was adjusted to 5.9. To reach the optimal digestive activity of enzymes in starch (12 u/mL) [21], 5.8 cc of the enzyme solution with 207 u/mL of enzyme were added to 100 cc of the starch substrates (150 g/L). Then, the medium was hydrolyzed at 65 °C for 3 h and 15 min to produce reducing sugar [22]. As can be seen in Figure 1, the effect of the extracellular enzymes of B. amyloliquefaciens ATCC 23350 on starch varied depending on the plant sources.

The highest amount of reducing sugar (102.57 g/L) was obtained from polysaccharides of sprouted wheat. According to previous reports, a thousand-fold increase of amylase and a combination of alpha- and beta-amylase efficiently breaks down starch in sprouted wheat [23]. Under these conditions, the efficiency of microbial enzymes greatly increased, so high degradation of starch and the maximum amount of reducing sugar could be seen in this medium. The content of reducing sugar obtained from millet starch (57.50 g/L) was higher than that from wheat extract (51.62 g/L). Research has shown that the surface-to-volume ratio of granules varies inversely with the rate of enzymatic hydrolysis of the granules. The shape of millet and wheat granules is polyhedral and lenticular, respectively. The surface-to-volume ratio of polyhedral granules is higher than that of lenticular ones, so it could be speculated that alpha enzymes from B. amyloliquefaciens ATCC 23350 have more surface potential for the hydrolysis of starch, as reported by Tester et al. [24]. Starch with high concentrations of amylase is resistant to enzymatic hydrolysis [25,26]. The amylose content in millet (19.8%) is less than that in wheat (28.8%), so the reducing sugar produced from millet by enzyme activity was higher than that from wheat starch. The results showed that the amount of reducing sugar produced from wheat polysaccharides by Bacillus enzymes is higher than that from oat starch. Oat starch is strongly bound to beta-glucan

![Figure 1. Reducing sugar produced by B. amyloliquefaciens ATCC 23350 from different carbon resources in the growth medium.](image)

| Starch source | Wheat germ | Millet | Wheat | Oat | Corn | Potato | Rice | Acanthophyllum braeuctum |
|--------------|------------|--------|-------|-----|------|--------|------|--------------------------|
| Sugar content (g/L) | | | | | | | | |
| 0 | 20 | 40 | 60 | 80 | 100 | 120 | | |

Table 1. Effect of glycerol and glucose as carbon sources on the lipid content (%) and dry weight biomass (%) of yeast isolates from fish gills.

|                | Trout | Carp | Zander | Mullet |
|----------------|-------|------|--------|--------|
| Glucose Lipid  | 27 ± 0.3 | 22 ± 0.8 | 21 ± 0.6 | 16 ± 0.4 |
| Biomass        | 0.5 ± 0.04 | 0.36 ± 0.16 | 0.74 ± 0.10 | 0.4 ± 0.15 |
| Glycerol Lipid | 25 ± 1.2 | 22 ± 1.0 | 22 ± 1.0 | 10 ± 0.8 |
| Biomass        | n        | 0.50 ± 0.07 | 0.15 ± 0.08 | 0.4 ± 0.11 |

n, negligible amount


Table 2. Profile of saturated fatty acids produced by Mortierella alpina CBS 754.68 and Wickerhamomyces siamensis SAKSG grown in media with different starch/carbon sources (% w/w).

|          | Wheat | Wheat germ | Rice | Corn | Gel | Millet | Potato |
|----------|-------|------------|------|------|-----|--------|--------|
|          | M. alpina | M. siamensis | M. alpina | M. siamensis | M. alpina | M. alpina | M. alpina | M. alpina | M. siamensis | M. alpina | M. siamensis | M. alpina | M. siamensis |
| C6:0     | 10.56 ± 0.01 | 5.08 ± 0.7 | 7.42 ± 0.9 | 3.07 ± 0.6 | 5.36 ± 0.7 | 4.76 ± 0.03 | 14.77 ± 0.2 | 6.70 ± 0.00 | 19.36 ± 1.0 |
| C8:0     | 6.63 ± 0.4 | 4.00 ± 0.05 | 5.42 ± 1.0 | 1.51 ± 0.02 | 0 | 2.67 ± 0.06 | 0 | 6.43 ± 0.9 | 0 | 6.78 ± 1.2 |
| C10:0    | 11.34 ± 0.02 | 4.94 ± 0.08 | 7.88 ± 0.4 | 2.27 ± 0.06 | 0 | 5.04 ± 0.09 | 0 | 10.44 ± 0.05 | 0 | 9.71 ± 0.06 |
| C12:0    | 14.80 ± 0.2 | 6.63 ± 0.06 | 10.07 ± 0.02 | 2.79 ± 0.05 | 0 | 6.56 ± 0.09 | 0 | 14.22 ± 0.7 | 0 | 13.58 ± 0.3 |
| C14:0    | 9.15 ± 0.3 | 3.29 ± 0.3 | 3.96 ± 0.01 | 3.48 ± 0.9 | 6.19 ± 0.05 | 0 | 3.54 ± 0.1 | 0 | 3.62 ± 0.5 | 0 | 3.32 ± 0.07 |
| C15:0    | 8.56 ± 0.4 | 4.27 ± 0.05 | 7.04 ± 0.4 | 0 | 1.37 ± 0.05 | 2.79 ± 0.7 | 1.44 ± 0.01 | 0 | 1.22 ± 0.5 | 0 | 1.32 ± 0.07 |
| C16:0    | 15.34 ± 0.3 | 6.19 ± 0.5 | 13.37 ± 0.6 | 15.06 ± 0.05 | 14.36 ± 0.06 | 9.61 ± 0.00 | 12.21 ± 0.03 | 10.64 ± 0.07 | 12.57 ± 0.05 | 12.36 ± 0.08 | 15.53 ± 0.1 | 7.21 ± 0.1 | 11.71 ± 0.07 | 5.94 ± 0.1 | 11.46 ± 0.1 | 6.26 ± 0.6 |
| C17:0    | 0.55 ± 0.05 | 0.38 ± 0.04 | 0.41 ± 0.07 | 0 | 0 | 0 | 0.19 ± 0.5 | 0 | 0 | 0.56 ± 0.07 | 0 | 0.67 ± 0.7 |
| C18:0    | 3.30 ± 0.1 | 2.28 ± 0.9 | 3.70 ± 0.5 | 5.17 ± 0.6 | 2.29 ± 0.1 | 2.68 ± 0.08 | 2.57 ± 0.5 | 2.67 ± 0.5 | 2.44 ± 0.1 | 2.09 ± 0.09 | 3.06 ± 0.7 | 2.19 ± 0.6 | 2.88 ± 0.1 | 2.85 ± 0.7 | 2.82 ± 0.05 | 3.90 ± 1.1 |
| C20:0    | – | – | – | – | 0.39 ± 0.5 | 0.43 ± 0.01 | 0 | 0.94 ± 0.4 | 0.51 ± 0.2 | 0 | 0.23 ± 0.9 | 0 | – |
than 20% w/w lipids; they were considered as oleaginous species. The lipid content was the highest in the yeast isolate from trout (Table 1). This isolate was selected for further study. The results showed that the yeast isolate obtained from zander produced higher amounts of biomass than the other tested isolates (Figure 1).

Li et al. [16] reported that glycerol as a carbon source had a significant effect on the lipid production in some yeast species. Therefore, glucose was replaced by glycerol (30%). The results indicated that glycerol had a negligible effect on the biomass production, but this substrate supported better conditions for lipid production than glucose substrate from gill yeast of carp and zander fish species. However, due to the lowest biomass production by glycerol, glucose was selected for efficient medium for lipid production (Table 2).

**Molecular identification of the microbial species**

In this step, the non-transcribed spacer 2 sequence of the yeast isolate was compared with the ribosomal sequence of other similar species using in the NCBI database. The phylogenetic tree that was drawn with Mega5 software (Figure 2) verified that the isolated species was very similar to *Wickerhamomyces* sp., and therefore it was named *Wickerhamomyces siamensis* SAKSG (Table 1).

**Fatty acids profile**

There were different amounts of fatty acids in the media (Tables 2 and 3). In *Mortierella alpina*, C15 was only seen in the oil of the millet and wheat media. Also, C14 was found in potato, rice and sprouted wheat carbon media. Other saturated fatty acids were seen in all carbon sources media. The dominated fatty acid was palmitic acid (Table 2). The highest amounts of linoleic acid, oleic acid and arachidonic acid were observed in the media with corn (41.873%), rice (30.061%) and potato (27.054%) as the carbon source, respectively (Table 3). The fatty acid profile of *Mortierella alpina* showed that the arachidonic acid content of the corn medium was the lowest (10%), whereas the linoleic acid content was the highest (42%). To our knowledge, this is the first report of such an amount of linoleic acid in the *Mortierella* lipid profile. Overall, the fatty acid profiles of *Mortierella alpina* lipids were considerably changed in different media; these patterns had not been previously observed except in species altered by genetic engineering methods. Jareonkitmongkol et al. [34] reported that a considerable amount of linoleic acid was observed by silencing of the delta-6 gene. Thus, there may be some inhibitor factors in the medium which caused the accumulation of linoleic acid. After the corn medium, the *A. bracteatum* medium had the highest biomass content, which may be due to the highest amounts of glucose sugar added to the media and the lowest amount of the medium starch. The highest amount of arachidonic acid (25%) was obtained due to the high amount of added glucose and the low amount of the medium starch. Hence, the non-hydrolyzed starch could be considered as a negative factor on arachidonic acid production. There was no comparative difference between the amount of reducing sugar and non-hydrolyzed starch of the millet and wheat starch media, so the arachidonic acid content in these media was similar. This result indicated that the reducing sugar and non-hydrolyzed starch content could greatly affect the arachidonic acid production by *Mortierella alpina*. The rice medium had minimum starch decomposition. Thus, its reducing sugar was mainly enriched in glucose; however, the medium had a high percentage of

**Figure 2.** Phylogenetic tree of the unknown yeast isolate with 11 similar species based on nearest neighbour interchange analysis of the non-transcribed spacer 2 region (bootstrap level: 1000 pseudoreplications).
Table 3. Profile of unsaturated fatty acids produced by Mortierella alpina CBS 754.68 and Wickerhamomyces siamensis SAKSG grown in media with different hydrolysed starch/carbon sources (% w/w of lipid).

| Fatty Acid | M. alpina | W. siamensis | M. alpina | W. siamensis | M. alpina | W. siamensis | M. alpina | W. siamensis | M. alpina | W. siamensis |
|------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|
| C12:0      | 5.93 ± 0.01 | 2.33 ± 0.03  | 4.02 ± 0.09 | 7.79 ± 0.08  | 1.0 ± 0.05 | 0            | 2.15 ± 0.05 | 0            | 2.38 ± 0.01 | 0            |
| C14:0      | 5.17 ± 0.01 | 6.01 ± 0.01  |            | 2.27 ± 0.1   | 0.77 ± 0.08 | 0            | 0.15 ± 0.2  | 0            | 0.58 ± 0.2  | 0            |
| C16:0      | 4.28 ± 1.0  | 2.80 ± 0.9   | 2.83 ± 0.5  |              |            | 0.32 ± 0.1   | 0.21 ± 0.1  | 0.70 ± 0.5   | 0.43 ± 0.1  | 0.78 ± 1.0   |
| C18:0      | 0.59 ± 0.01 | 3.66 ± 0.04  |            |              |            | 0.31 ± 0.1   |              |              |              |
| C18:1      | 24.19 ± 0.1 | 7.18 ± 0.3   | 24.13 ± 0.2 | 23.60 ± 0.6  | 30.06 ± 0.25| 20.69 ± 0.9  | 24.88 ± 0.8 | 25.85 ± 1.0  | 24.12 ± 0.5 | 20.04 ± 0.9  | 19.12 ± 0.3 | 14.40 ± 1.0 | 28.44 ± 0.1 | 27.28 ± 0.3 | 18.61 ± 0.3 | 4.30 ± 0.4  | 17.76 ± 0.05 | 4.42 ± 0.1  |
| C18:2      | 6.16 ± 0.2  | 0.30 ± 0.8   | 15.78 ± 0.3 | 1.72 ± 0.7   | 7.65 ± 0.05| 0.48 ± 0.6   | 7.80 ± 0.2  | 0.75 ± 0.05 | 11.69 ± 0.1 | 1.91 ± 0.6   | 5.98 ± 0.3  | 0.81 ± 0.8  | 9.96 ± 0.1  | 0.35 ± 0.6  | 17.07 ± 0.3 | 0.24 ± 0.1  |
| C20:2      | 0.42 ± 0.4  |              | 0.40 ± 0.08 | 0.22 ± 0.1   |              | 0.98 ± 0.04 |            |              |            |              |            |            |            |            |            |            |
| C20:3(w3)  |              |              |              |              | 0.12 ± 0.05 |            |            |              |            |              |            |            |            |            |            |            |
| C20:3(w9)  |              |              |              |              | 0.05 ± 0.7  |            |            |              |            |              |            |            |            |            |            |            |
| C20:4      | 24.57 ± 0.4 |              | 16.46 ± 0.6 | 0.53 ± 0.03  | 21.07 ± 0.4 | 0.47 ± 0.5  | 10.65 ± 0.13| 0.19 ± 0.07 | 25.43 ± 0.1 | 0.50 ± 0.03  | 25.05 ± 0.7 | 0.60 ± 0.1  | 27.05 ± 0.1 | 0.43 ± 0.06 | 24.94 ± 0.7 | 0.51 ± 0.05 | 0            |            |            |            |
| EPA        |              |              |              |              |              |            |            |              |            |              |            |            |            |            |            |            |            |            |
| DHA        |              |              |              |              |              | 0.76 ± 0.12| 0.20 ± 0.07 | 0.18 ± 0.3  |              |            |              |            |            |            |            |            |            |

EPA, eicosapentanoic acid; DHA, docosahexaenoic acid

The results showed that the formulated media greatly affected the weight of biomass production by Mortierella alpina, supported the highest amount of dry weight of biomass production by Mortierella alpina. These results are in agreement with Liu et al. [6], who reported that glucose and starch together in the medium highly affect biomass production. In comparison, the medium with other shake-flask studies [33–44] on this fungal species (Table 4), we achieved higher dry weight biomass of Mortierella alpina.

The results revealed that the formulated media greatly affected the M. alpina biomass (Figure 3). The highest and lowest amounts of biomass were obtained in the best medium for reducing sugar production by M. alpina amylase, supported the highest amount of dry weight of biomass production by Mortierella alpina. These results are in agreement with Liu et al. [6], who reported that glucose and starch together in the medium highly affect biomass production. In comparison, the medium with other shake-flask studies [33–44] on this fungal species (Table 4), we achieved higher dry weight biomass of Mortierella alpina.
sprouted wheat. This medium supported high biomass production by *Mortierella alpina*. The formulated media favoured the production of various kinds of fatty acids by the oleaginous yeast isolate and *Mortierella alpina*. The considerable effects of the media on the fatty acids profile indicated that the predominant fatty acids of microbial oils change depending on the medium composition. These trends could be used to produce various health-beneficial fatty acids from definite strain based on the medium conditions to meet the industrial needs.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Table 4.** Reported dry weight biomass of *M. alpina.*

| Microorganism              | Dry weight biomass (g/L) | References |
|----------------------------|--------------------------|------------|
| Mortierella alpina CBS 754.68 | 37                       | This study |
| Mortierella alpina LPM301   | 25.61                    | [39]       |
| Mortierella alpina M6       | 31.20                    | [40]       |
| Mortierella alpina SC9      | 30.51                    | [41]       |
| Mortierella alpina M6       | 22.50                    | [42]       |
| Mortierella alpina DSA-12   | 20–25                    | [43]       |
| Mortierella alpina HK1      | 36.20                    | [44]       |

**Figure 3.** Dry weight biomass of *Mortierella alpina* CBS 754.68 in media with different carbon sources.

**Conclusions**

The effects of *B. amyloliquefaciens* ATCC 23350 enzymes on starch varied according to the type of starch source. A high content of reducing sugar and hydrolyzed starch was achieved in the medium supplemented with biomass ([Figure 3](#figure3)). Finco et al. [13] showed that the reducing sugar content has a significant effect on biomass production. When considering the structural fatty acids based on Gray et al. [45], oleic acid and linolenic acid are the prevalent fatty acids that exist in microorganism membranes. The biomass increase was associated with accumulation of these types of structural fatty acids, so the highest amount of linolenic acid (16% of the lipid content) was observed in the sprouted wheat medium. Considering the biomass production, the corn medium was not appropriate for enzyme decomposition by *B. amyloliquefaciens*. Thus, the lowest reducing sugar content with a high amount of non-decomposed starch was obtained in this medium. Consistent with previous research [46,47], the protein content was more important for biomass accumulation than the carbon sources. The high protein content of corn, which has essential amino acids, significantly affected the biomass accumulation, despite the high non-decomposed starch content.
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