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The rise in global temperature is a direct reflection of the problem of cellulosic pollution. Hence, it was necessary to find cheap, safe and effective ways to convert these cellulosic wastes into useful products. The present manuscript aims to introduce an effective and applicable strategy for safe conversion of agricultural cellulosic wastes into useable high protein, carbohydrate and lipid biomass. The current study presents a miniature model for converting the cellulosic waste of straw (residues) into a living biomass containing high amounts of pigments, proteins and lipids stored in the algae, *Chlorella vulgaris*. Two soil microorganisms with a high ability to cellulose degradation were selected, isolated, biochemically and molecularly identified as *Aspergillus terreus* and *Brevibacillus borstelensis*. The obtained results showed the highest recorded sugar productivity of 648.1µg/ml on the 8th day of incubation with *A. terreus* (untreatment straw), and 814 µg/ml on the 4th day with µg/ml *B. borstelensis* (pretreatment straw). The mixotrophic nutrition of *Chlorella vulgaris* µg/ml grown on degraded straw by both organisms showed significant increment (enhancement) in growth, pigment, protein and lipid content compared to those produced by the alga cultivated on the KC nutrient medium. It could be concluded that treated cellulosic wastes can be used as safe mixotrophic nutrition for microalgae to enhance the productivity of biomass and other biochemical parameters.

Keywords: *Brevibacillus borstelensis, Aspergillus terreus, Chlorella vulgaris,* cellulosic wastes, reducing sugar

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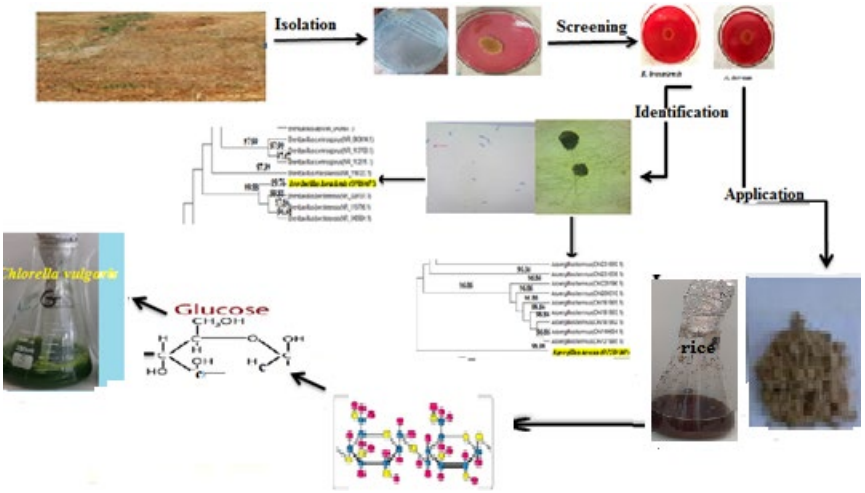
GRAPHICAL ABSTRACT



INTRODUCTION

Wastes in general terms are defined as unwanted and undesirable residues. Industrial, agricultural, municipal solid trash and hazardous wastes are the four main categories of wastes (Halleux, 2022). Egypt produces approximately 50 to 60 thousand tons of solid wastes daily that reach about 22 million tons per year, according to the Solid Waste Management Regulatory Authority (SWMRA) (El Masry et al., 2022). Agricultural waste is generated day by day due to increasing the agriculture production. Most of these wastes are misused either by burning and/or disposing with unsuitable methods. This increases the greenhouse gases (GHGs) emission in the earth's atmosphere (Elbasiouny et al., 2020). Rice is the main cereal crop for over 50% of the world's population (Elbasiouny et al., 2020). Agricultural residues consist mainly of cellulose and lignin, which jointly represents 85–90% of the dry matter content; the remaining 15–10% includes simple sugars, starch, fat, ash, wax, essential oils, gums, pectin, tannins, and among other substances (Rodríguez, 2010). Cellulose $(C_6H_{10}O_5)_n$ is a polysaccharide consisting of a linear chain of several hundred to many thousands of $\beta(1 \rightarrow 4)$ linked d-glucose units (Gupta et al., 2019). Typically, it is assumed that fungi are the major decomposers of complex plant biopolymers in soils (Darwesh et al., 2020). Fungi are very important organisms that inhabit all types of soil. Cellulolytic fungi are one of the fungi that play a role in the process of

decomposition and degradation of organic matter. Cellulolytic fungi decompose organic material containing cellulose by producing cellulase enzymes. Common fungi producing cellulase enzymes include the genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Acremonium*, *Chaetomium*, *Rhizopus* and *Curvularia* (Tuli et al., 2015; Abou-Zeid et al., 2020; El-Halmouch et al., 2024).

Bacteria are being widely explored for cellulases production because of their rapid growth, expression of multi-enzyme complexes, stability at extremes of temperature and pH, lesser feedback inhibition, capacity to colonize a wide variety of environmental niches, and ability to withstand varieties of environmental stress (Maki., et al., 2009). Soil bacteria secrete several enzymes which degrade lignocellulosic biomass. These enzymes are commonly produced by some bacterial genera such as *Cellulomonas* sp, *Pseudomonas* sp. that are widely used in industrial applications (Chukwuma et al., 2021).

The most-used carbon sources to grow mixotrophic microalgae are glucose, glycerol, and acetate. Glucose has been the most used organic carbon source for microalgae cultivation because it produces more energy per mole than other substrates. This monosaccharide is also abundant in sugarcane molasses and syrups that result from vegetable pulp extraction, such as carob pulp, which has been used in media formulation for heterotrophic microalgae growth (Taborda et al., 2021). Some wastes contain organic and inorganic components that may serve as nutrients for algal growth, decreasing the culture media cost and, thus, the overall process costs (Mohammad et al., 2023). Most studies on microalgae waste treatment use autotrophic and mixotrophic microalgae growth showed several benefits over the others, such as higher organic carbon load tolerance, intracellular products production, and stability in production all year round, regardless of the location and climate (Silva et al., 2021). Greater cell masses are obtained due to the higher carbon source energy density when compared to CO_2 (Perez-Garcia et al., 2011). El-Sheekh et al., (2012) reported that the growth, carbohydrate and protein content of *Chlorella vulgaris* and *Scenedesmus obliquus* were enhanced through heterotrophic and mixotrophic nutrition of decomposed cellulosic wastes by two fungi (*Pleurotus ostreatus* or *Trichoderma viride*).

Algal mixotrophic nutrition starts to be the effective strategy for energy preservations (El-Mohsnawy et al., 2020; El-Sheekh et al., 2020). Since many wastes contain high carbohydrate contents, providing algal growth media with sugar containing residues enhances heterotrophic nutrition that consequently promotes the algal growth (El-Sheekh et al., 2020; Taborda et al., 2021). Presenting an effective and applicable model for the environmentally friendly conversion of rice wastes into reduced sugar by *Aspergillus terreus* and *Brevebacillus brostelensis* were the aim of the current study. The resulting products were utilized to feed *Chlorella vulgaris* as mixotrophic nutrition, which increased its biomass output and growth.

MATERIALS AND METHODS

Isolation and cultivation conditions of cellulose-digested microorganisms

Dry soil samples were collected from the agricultural area. One gram of dried soil sample was suspended in 9 ml of sterile distilled water. After vigorous shaking, the suspension was subjected to serial dilution. 100 µl of each dilution was spread on potato dextrose agar plates to isolate fungi, and nutrient agar plates to isolate bacteria. Then the plates were incubated for 7 days at 30°C (Hankin and Anagnostakis 1977; Atalla et al., 2023a). For selection the best cellulose digesting strain, pure colonies were transferred to agar basal salt media containing 1% carboxymethyl cellulose (CMC) and incubated for 48 hours at 30°C. After incubation period, 0.1% Congo red solution was added and counterstained with 1M NaCl for 15–20 min (Hankin and Anagnostakis 1977).

Phenotypic, biochemical and molecular identification of cellulose-digesting strains

Phenotypic features of the purified cellulose hydrolyzed strains were investigated by determining the texture, aerial mycelium and growth of colony on potato dextrose agar plates for fungal isolate and nutrient agar plates for bacterial isolate (Sevvel *et al* 2016). Bacterial strain was undergoing gram stain and microscopic examination (Shiriling and Gottlieb, 1966). Biochemical tests, H_2S production, indole test, casein hydrolysis, gelatin hydrolysis, amylase (starch hydrolysis test), urea hydrolysis, phosphatase test, nitrate reduction test, catalase test and oxidase test were performed for biochemical identification (Atalla et al., 2023b). 16S rRNA was used for bacterial molecular identification (Norashirene *et al.,* 2013) and 5.8S rRNA sequence analysis was used for fungal identification (Heeger *et al*. 2019). These were

performed by Sigma Company in Cairo city. The GeneBank accession number for the partial 16SrRNA gene sequence of bacteria and 5.8S rRNA for fungal strain were performed.

Cellulosic Materials Preparation

Rice cellulosic materials (Giza 179) were collected from cultivated lands, soaked in distilled water for 24 hours, then washed with running water for an hour and dried in air (Fatmawati, et al., *2012*). The dried cellulosic materials were cut to 5 cm fragments and milled using mixer machinery. Finally, it was sieved to obtain the particle in appropriate size (Fatmawati, et al., *2012*). Concerning pretreated samples, 60 grams of cellulosic material were suspended in 800 ml NaOH (11%) and boiled at 100 °C for 20 min. After treatment, solid materials were washed for neutralization and dried (Fatmawati, et al., 2012).

Biodegradation of rice husk and cultivation condition

100 ml of basal salt liquid medium (El-Tanash, et al., 2010) free carbon source was added in 250 ml conical flask. Nitrogen sources were supplied as 2g of $NaNO_3$ for bacteria and 2.5g of NH_4NO_3 for fungus was used (El-Tanash, et al., 2010). The experiment was designed as 3 groups for each organism, *B. borstelensis* or *A. terreus*. The 1st group (GI) was supplied by 3.5 g CMC, the 2nd group (GII) was supplied by 3.5 g non-hydrolyzed rice husk and the 3rd group (GIII) was supplied by 3.5 g hydrolyzed rice husk (purchased from cultivated area). Each group was represented in three replicates. After sterilization and cooling each flask was inoculated with 1 ml of *B. borstelensis* or *A. terreus* under septic conditions. Flasks were shaken on an orbital shaker at 130 rpm for 8 and 4 days at 30° C and 35° C, pH values were 6 and 7 for *Aspergillus terreus,* and *Brevibacillus borstelensis,* respectively. Determination of the producing reducing sugar concentration was performed in certain periods, (3, 7, 10, 14, 17, 20 and 23 days) using Dinitrosalycalic acid technique (DNS) (Miller 1959).

Cultivation of C. vulgaris on cellulosic digested rice husks

After microbial cultivation, microbial cultural filtrates were collected via centrifugation at 8000 g for 20 min. 100 ml Filtrates of each treatment were divided into 2 parts. The 1st part was autoclaved and signed as sterilized filtrate and the 2nd part was non-sterilized. All treatments were inoculated by 5 ml of freshly cultivated cultures of *Chlorella vulgaris* reaching OD_{680nm} 0.05. Kessler and Czygan medium (KC medium) was used as control (Kessler and Czygan, 1970). Cultures were incubated at 25±2°C, continuous white fluorescence illumination of 80 $\mu Em^{-2}s^{-1}$ and satirized air bubbling. Algal growth was monitored by optical density at 680 nm (El-Mohsnawy et al., 2020). Reducing sugar concentration was monitored using DNS method (Miller 1959).

Estimation of pigment content

2 ml algal culture was centrifuged for 5 min at 5000g. Pellets were suspended in 2 ml acetone 99%. Vortex was performed for 2 min, till pellets become pale in color. After centrifugation at 8000 g for 5 min, absorbance of filtrate was measured at 470, 661.6 and 644.8 nm using JASCO V-730 spectrophotometer (Lichtenthaler et al., 1984). Chlorophylls and carotenoids concentration were estimated according to the following equations were used:

Chl. *a* = 11.24 *A 661.6 - 2.04 *A644.8 (µg/ml)
Chl. *b* = 20.13* A 644.8 - 4.19 *A661.6 (µg/ml)
Car = (1000 *A470 - 1.90* Chl *a* - 63.14* Chl *b*) / 214 (µg/ml)

Estimation of protein content

Protein extraction was performed according to Price (1965). 5mg of each air-dried *Chlorella vulgaris* was suspended and vortexed in 200 µL (24% w/v) trichloroacetic acid (TCA). After homogenizing, mixture was incubated in a water bath at 95 °C for 15 minutes. Protein extract was cooling down at room temperature and diluted to 600 µL using 24% (w/v) TCA. Mixture was centrifuged at 15,000g at 4 °C for 20 minutes (Microcentrifuge LC-04S - A042012 China). Protein quantification was performed according to Bradford (1976) and Stoscheck (1990). Extracted protein was mixed with equal volume of Coomassie Brilliant Blue Dye G-250 (CBBG) and left for 5 min before measuring the absorbance at 595 nm. Protein concentration was estimated by using standard solutions of a protein (Bovine serum albumin).

Estimation of lipid content

Algal lipid productivity was estimated according to SPV method (Van Handel, 1985). 30 mL of *C. vulgaris* cultures were harvested by centrifugation at 5000 g for 10 min. Pellets were dried after being washed twice with distilled water. 0.5 ml of a 1:1 chloroform-methanol mixture was used to suspend 0.01 g of dried algae samples, which were then gently shaken. Then, 0.25 ml of the filtrates were transferred to a clean glass tube, and the solvents were evaporated in a water bath set at 100 °C. After cooling, 0.1 ml of sulfuric acid was added, and mixture was heated at 100 °C for 10 minutes. Samples were cooled before

adding 2.5 ml of vanillin reagent. After half an hour, a reddish color was observed and absorbance was measured at 525 nm using spectrophotometer (Jasco V-730 UV-Visible Spectrophotometer). The lipid concentration was estimated by using a standard curve of soybean oil under the same conditions.

Statistical Analysis

Data presented on the average of three replicates are obtained from their independent experiments**.** Minitab 19 software was used. ANOVA one-way was carried out.

RESULTS

Isolates selection

Data of several microbes able to undergo biodegradation of cellulose indicated that all the isolates were grown on culture medium containing CMC (Carboxymethyl cellulose) as a sole carbon source at 37 °C. Biodegradation assay of examined microbes was observed as 26.7±2.0 mm and 29±0.2 mm for *Aspergillus terreus*, *Brevibacillus borstelensis*, respectively (Figure 1).

Morphological characterizations of isolated microbes

Obtained results emphasis that bacterial isolate is a gram positive, aerobic, rod-shaped endospore forming bacterium. Also, observed colonies were as irregular in shape, Smooth in surface, Creamy in color, Mucoid in opacity, raised in elevation and the margin was irregular (Figure 2). Fungal isolate colonies appeared as cinnamon brown in color with whitish margin. By investigation of the fungal mycelium using light microscope, it appeared as biseriate conidiophores and unseptate hyphae (Figure 3)

Biochemical and molecular characterization of bacterial isolate

The obtained biochemical tests revealed a positive result against the detection of amylase (starch hydrolysis test), nitrate reduction, catalase, and oxidase, but a negative result against H_2S production, Indole test, Casein hydrolysis, Gelatin hydrolysis, Urea hydrolysis, and Phosphatase test. The 16S rRNA gene amplification produced approximately nucleotides length of 1419 bp (Fig 4). The resulting sequence was analyzed using an online database (NCBI) and compared to other bacterial isolates. It revealed that a 99.76 % similarity with *Brevibacillus borstelensis strain Logan B4029* according to NCBI GeneBank. The GeneBank accession number for the partial 16SrRNA gene sequence of *B. borstelensis* strain Logan B4029 was OP860677.

Molecular identification of fungal isolate

The fungal isolate was identified according to a molecular biology protocol by DNA isolation, amplification (PCR) and sequencing of the 5.8S ribosomal RNA was 693 bp. Blast search of the 5.8S partial sequences revealed that the isolate was closely related to *Aspergillus terreus strain 9207* with similarity of 96.90%, as shown in Fig 5. The GeneBank accession number for the partial 5.8S rRNA gene sequence of *Aspergillus terreus* strain 9207 was OP580169.

Biodegradation of rice cellulosic wastes

The efficiency of *A. terreus* to bioconversion the cellulosic wastes of rice husk and CMC as control into reducing sugar for 18 days incubation period is shown in Table 1. The recorded reducing sugar production in the case of untreated wastes was higher than those in the case of pretreated wastes. A dramatic increase of both cases was observed reaching the maximum values at the 8th day of the incubation period. The highest reducing sugar values obtained by *A. terreus* in untreated rice wastes were 648.1 µg /ml. It represented a relatively half of those obtained by CMC culture (1174 µg /ml).

The efficiency of *B. borstelensis* for bioconversion of rice husk, and CMC as control into reducing sugar through 18 days incubation period is shown in Table 2. The recorded reducing sugar production in the case of pretreated wastes was higher than those in the case of untreated wastes. Obtained results showed that the highest reducing sugar production was obtained on the 4th day and then decreased. Maximum reducing sugar production was observed at the 4th day for all treatments. The highest reducing sugar values were obtained by *B. borstelensis* in pretreated rice wastes were 814.7 µg /ml. They represented approximately 73% of those obtained by CMC culture (Table 2).

Growth estimation of C. vulgaris cultivated on microbial filtrates

Chlorella vulgaris, cultivated on rice filtrate of *A. terreus and B. borstelensis* showed high growth based compared to CMC culture or KC medium. The maximal growth of *C. vulgaris* was observed after 17th day in case of *A. terreus*. The growth of *C. vulgaris* in non-sterilized and sterilized *A. terreus* filtrates were increased 1.24 and 1.14 folds, respectively compared

Table 1. Influence of incubation period of different wastes treatment on reducing sugar production by *A. terreus* cultivated on rice wastes

Time (days)	Reducing sugar (µg /ml)		
	Rice		CMC control
	Untreated	Pretreated	
0	128.2^{g} ±1.0	68.5^{g} ±0.4	5.2^{d} ±0.3
3	283.3^{f} ± 0.6	86.7^{e} ±1.1	356.4^{c} ±0.5
6	355.3^{e} 0.3	174.1^{c} ±0.0	947.2^{b} ±0.6
8	**648.1^{a} ±1.5**	**231.3^{a} ±0.7**	**1174^{a} ±0.8**
10	577.1^{b} ±0.7	217.5^{b} ±0.5	989.5^{b} ±0.3
13	494.7^{c} ±0.4	131.6^{d} ±1.1	986.8^{b} ±1.6
16	428^{d} ±0.4	76.5^{f} ±0.1	974.6^{b} ±1.7

Table 2. Influence of incubation period on reducing sugar production by *B. borstelensis* cultivated on rice wastes.

Time (days)	Reducing sugar (µg /ml)		
	Rice		CMC control
	Untreated	Pretreated	
0	145.8^{e} ±0.3	172.9^{e} ±0.1	226.4^{d} ±5.3
2	254.7^{d} ±1.2	476.4^{d} ±1.6	816.8^{c} ±1.3
4	**716^{a}** ±0.2	**814.7^{a}** ±5.7	**1112^{a}** 4.1
7	523.9^{b} ±0.3	683.3^{b} ±1.1	1095^{ab} ±5.0
10	394.9^{c} ±0.5	607.2^{c} ±0.3	1084^{b} ±3.1

Table 3. Influence of incubation period on *Chlorella vulgaris* growth cultivated on rice filtrate collected from *A. terreus* and *B. borstelensis* liquid culture.

Time (days)	OD at 680 nm					
	Non-sterilized	Sterilized	Non-sterilized	Sterilized	CMC	KC.
	A. terreus		*B. borstelensis*			
0	0.93^{c} ±0.0	0.9^{d} ±0.0	1.02^{e} ±0.2	0.99^{h} ±0.0	0.47^{e} ±0.0	0.6^{h} ±0.0
3	2.3^{bc} ±0.0	1.7^{cd} ±0.2	2.86^{de} ±0.7	2.81^{g} ±0.1	1.29^{d} ±0.0	0.9^{g} ±0.0
7	2.8^{abc} ±0.2	2.03^{bcd} ±0.5	4.4^{d} ±0.4	3.36^{f} ±0.2	1.83^{c} ±0.2	1.7^{f} ±0.4
10	3.6^{ab} ±0.1	2.6^{abc} ±0.0	4.8^{c} ±0.7	4.57^{e} ±0.3	2.37^{b} ±0.1	2.01^{e} ±0.2
14	3.9^{ab} ±0.3	3.6^{ab} ±0.2	5.89^{b} ±0.5	4.74^{d} ±0.1	2.4^{b} ±0.0	3.19^{b} ±0.1
17	**4.6^{a}** ±0.3	**4.2^{a}** ±0.2	6.10^{b} ±0.9	5.1^{c} ±0.2	2.52^{b} ±0.0	**3.7^{a}** ±0.0
20	3.83^{ab} ±0.6	2.93^{abc} ±0.3	**7.84^{a}** ±0.6	**6.7^{a}** ±0.2	2.34^{b} ±0.1	2.89^{d} ±0.0
23	3.81^{ab} ±0.6	2.49^{bcd} ±0.3	7.2^{a} ±0.2	6^{b} ±0.6	**3^{a}** ±0.3	3.00^{c} ±0.1

to KC synthetic medium, (Table 3). In the case of CMC filtrate, the highest growth of *C. vulgaris* was obtained after 23 days of incubation, which was 0.81 folds more than that of KC medium. In contrast, the highest growth was observed using non sterilized and sterilized rice filtrate of *B. borstelensis* as a culture medium for *C. vulgaris* after 20 days of incubation. It represented two folds more than its growth in KC medium (Table 3).

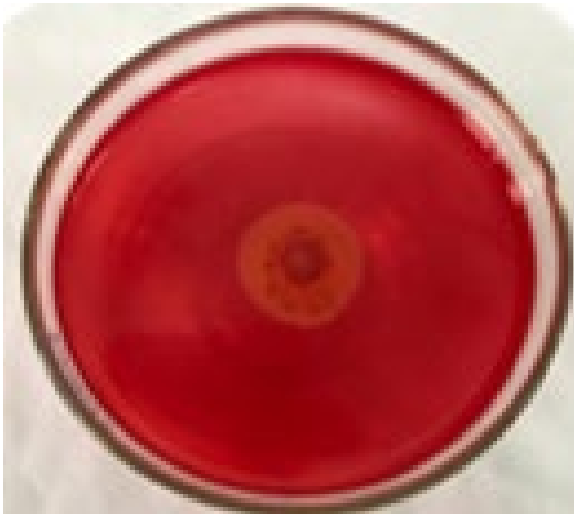
Pigment contents

Concerning chlorophyll, a, b and carotenoid pigment of *C. vulgaris*, data shown in Figure (6) illustrated significant differences of pigment content according to the used culture media. When *C. vulgaris* was grown on non-sterilized, sterilized and CMC of all tested microbes, the estimated pigment contents were higher than those of *C. vulgaris* grown in KC synthetic medium. The highest chlorophyll a (Fig. 6I) content was obtained in *C. vulgaris* cultivated on non-sterilized rice filtrate of *B. borstelensis*. On the other hand, the greatest values of chlorophyll b (Fig. 6II) were obtained in *C. vulgaris* cells grown on CMC filtrate of *A. terreus* and non-sterilized rice filtrate of *B. borstelensis*. On the other hand, the greatest values of carotenoid were obtained in *C. vulgaris* cells grown on all filtrates of *B. borstelensis* exceeding those of KC medium. The highest carotenoid content was obtained in *C. vulgaris* cells grown on non-sterilized and CMC greater than KC. The highest value of carotenoid content was obtained in *C. vulgaris* cells grown on sterilized rice filtrate was greater than that grown-on KC media (Fig. 6III).

Protein and Lipid contents

Protein and lipid content of *C. vulgaris* cultivated on microbial filtrates varied significantly compared with that of control. The highest value of protein content was observed in *C. vulgaris* cells grown on non-sterilized rice and CMC filtrate of *A. terreus.* They were 248.9 and 250.9 µg/ml, respectively.

B. brostelensis



A. terreus

Figure1. Determination of the cellulosic biodegradation activity of *B. borstelensis* and *A. terreus*. Cellulosic degradation is observed via a clear zone around some microbial colonies on CMC agar via following stained with Congo red dye.

They represented 1.4 and 2.3 folds compared to the control, respectively.In the case of *B. borstelensis*, the greatest protein content was observed on CMC filtrate. It was 236.8 µg/ml and represented 1.3 folds compared to the control. On the other hand, a significant value of lipid content of *C. vulgaris* cells grown on sterilized rice filtrate of *A. terreus*. The highest lipid content of *C. vulgaris* cells grown on non-sterilized filtrate of *B. borstelensis was* 255 µg/ml. Statistical analysis proved that there were no significant differences between the above results and that of control. The highest lipid content of *C. vulgaris* cells was achieved when it was grown on non-sterilized rice filtrates of *B. borstelensis* and sterilized filtrate of *A. terreus* (Figure 7).

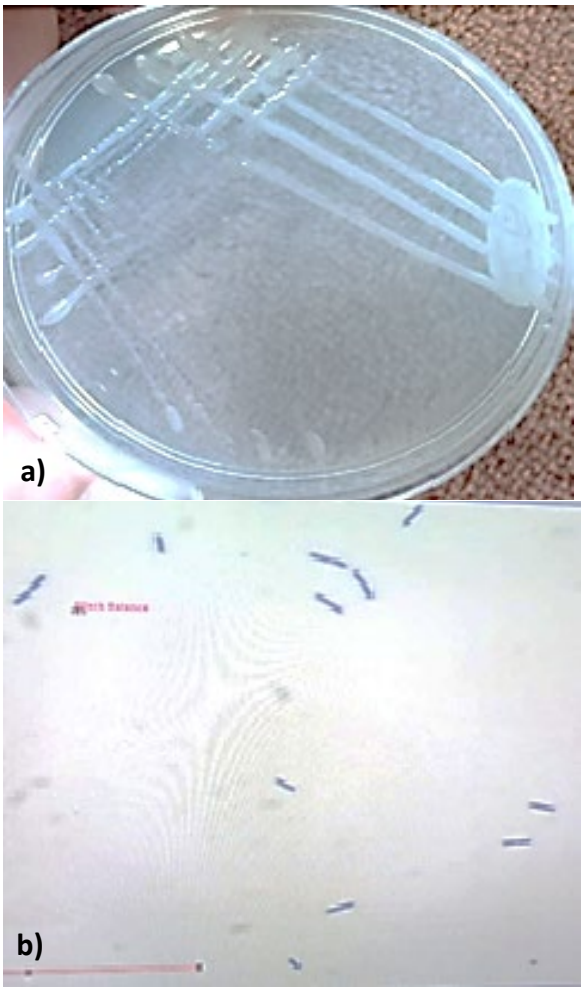


Figure 2. a) *B. borstelensis* colonies after 24 hours incubation on N.A medium, b) Microscopic examination of *B. borstelensis*

DISCUSSION

In present study, safe microbial factory for removing cellulosic wastes was established by isolation of two high cellulosic degradable microorganisms, *A. terreus,* and *B. borstelensis* from the soil and converted it into *C. vulgaris* biomass that considered the most efficient food for aquacultures. Bacterial and fungal isolates were identified according to the traditional protocols. Since several bacteria and fungi are capable of decomposing lignocellulose, they can be used as strong source for biocleaning from cellulosic wastes. They could secrete extracellular a variety of hydrolytic enzymes that can break down cellulose, hemicelluloses, and lignin (Saini et al., 2016).

Among the selected strains, the result showed that the degradation process of celluletic wastes highly depends upon the type of the celluletic materials, availability of nutrient sources and other environmental factors (Abubakar *et al* 2022).

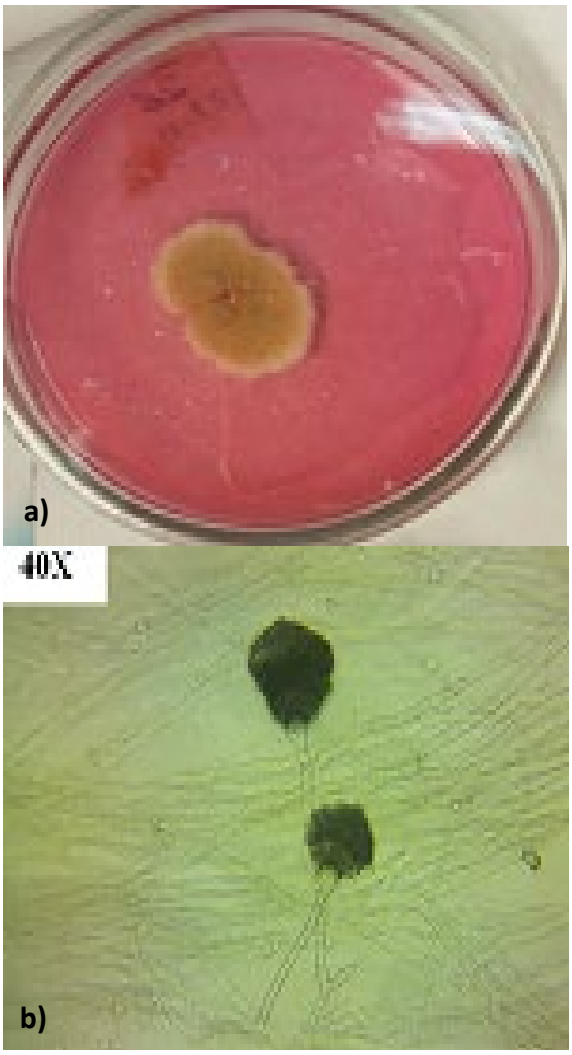


Figure 3. a) Colony of *A. terreus* on PDA plate of 5 days incubation, b) Microscopic examination of *A. terreus* of 3 days-old

C. vulgaris showed high reducing sugar consumption which formed from the activity of *A. terreus* and *B. brostelensis* rice filtrates. These results were due to the usage of reducing sugars as a carbon source through mixotrophic nutrition. Growth of *C. vulgaris* has been shown to outperform growth in KC medium in all filtrates of *B. borstelensis* and *A. terreus*, as growth measured at optical density of 680 nm. Many studies have proven that when microalgae and bacteria are cultured together, a shift in cellular composition occurs as well as increased growth (Tanabe et al., 2015; Amavizca et al., 2017; Xue et al., 2018). While Fukami et al., (1997) showed that the bacterial communities in aquatic environments influence the growth of microalgae by stimulatory and/or inhibitory effects. Cultivation of *C. vulgaris* with glucose influences metabolic carbon assimilation pathways, cells' size, quantity of storage materials (starch, lipids, protein) and cellular contents of chlorophyll, RNA, vitamins (Perez-Garcia et al., 2011). According to Mussatto et al., (2008), glucose is the main sugar produced by conversion of cellulose. Ili´c et al., (2023) reported that, fermentable sugars produced by enzymatic hydrolysis of cellulose could be a crucial biofuel production. Therefore, glucose is one of the most used carbon sources for most living cells and is used as a carbon and energy source in many heterotrophic cultures of microalgae (Vazhappilly and Chen, 1998; Jiang and Chen, 2000; Cheng et al., 2009). According to Shi et al., (1999) glucose can be considered the best organic carbon substrate for the growth of *Chlorella* that came in agreement of obtained results. All pigment contents of *C. vulgaris* were higher when it was grown on rice filtrates of *A. terreus* and *B. brostelensis* than those of *C. vulgaris* grown on KC medium. Chlorophyll *a* recorded the maximum value in case of non-sterilized rice filtrates of *B. borstelensis* and *A. terreus* than chlorophyll (b) and carotenoid. The formation of photosynthetic apparatus in *Chlorella* may be disturbed by the presence of organic substrates (Yang et al., 2000), resulting in a decreased production of photosynthetic pigments when compared with that obtained under photoautotrophic conditions.

Other publications (Ip et al., 2004; Kong et al., 2011) reported that, the higher content of chlorophylls obtained in the photoautotrophic culture confirms such observation when compared to mixotrophic cultured cells. Such observations with enhancement of chlorophyll biosynthesis by photoautotrophic *Chlorella* strains compared with that resulting from mixotrophic cells. The maximal protein content was obtained in *C. vulgaris* cells grown on non-sterilized rice and CMC filtrate of *A. terreus* and *B. borstelensis* filtrates than the control. Obtained results were like those reported by Velichkov and Sirakov (2018) who found that, the mixotrophic nutrition of *Chlorella vulgaris* by CO_2 and lactose significantly increased the protein content by 39%, while the mixotrophic nutrition of *Chlorella vulgaris* by CO_2 and glucose enhanced the protein content by 30.7% (Abreu et al., 2012). Also, Bajwa et al., (2016) observed a higher amount of protein under phototrophic conditions. The highest lipid content of *C. vulgaris* cells was achieved when it was grown on non-sterilized rice filtrates of *A. terreus or B. borstelensis*, compared with those of cells grown on KC medium. Montero-Sánchez *et al*. (2012) reported that, when *C. vulgaris* grows on an unfavorable environment; lipid production is enhanced, reporting up to 58% of the

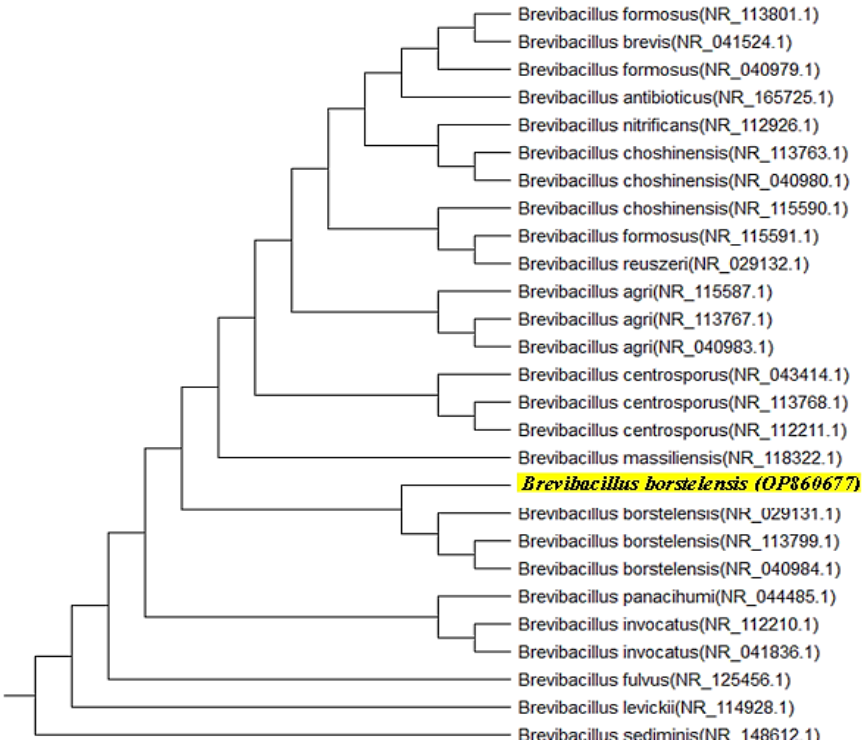


Figure 4. A phylogenetic tree of *Brevibacillus borstelensis strain Logan B4029*

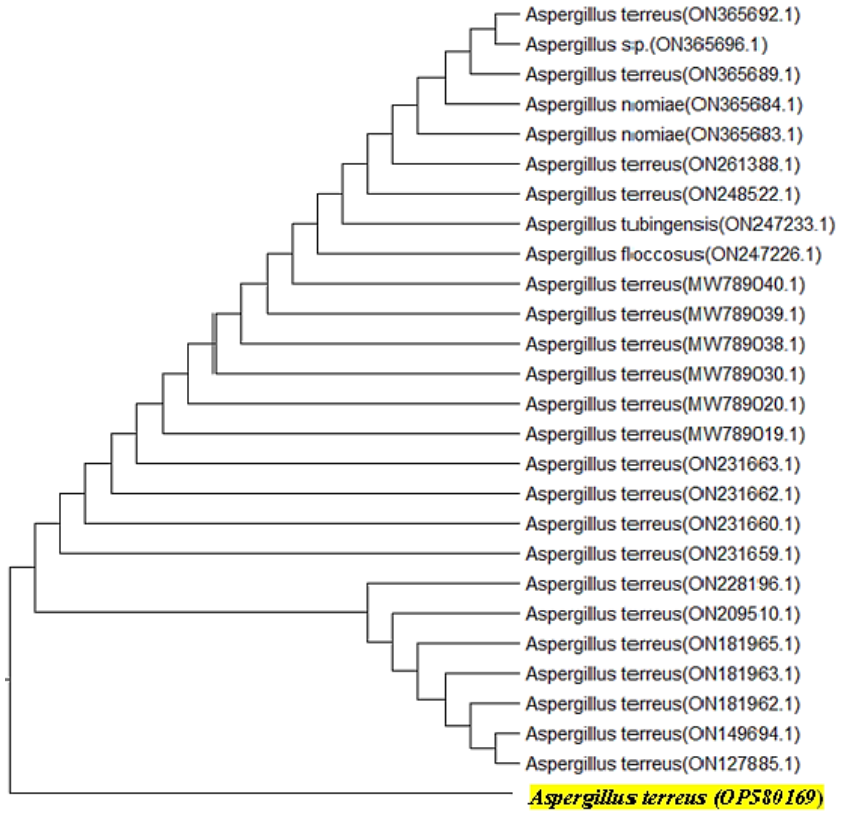


Figure 5. A phylogenetic tree of *Aspergillus terreus strain 9207*against other fungi neighbors.

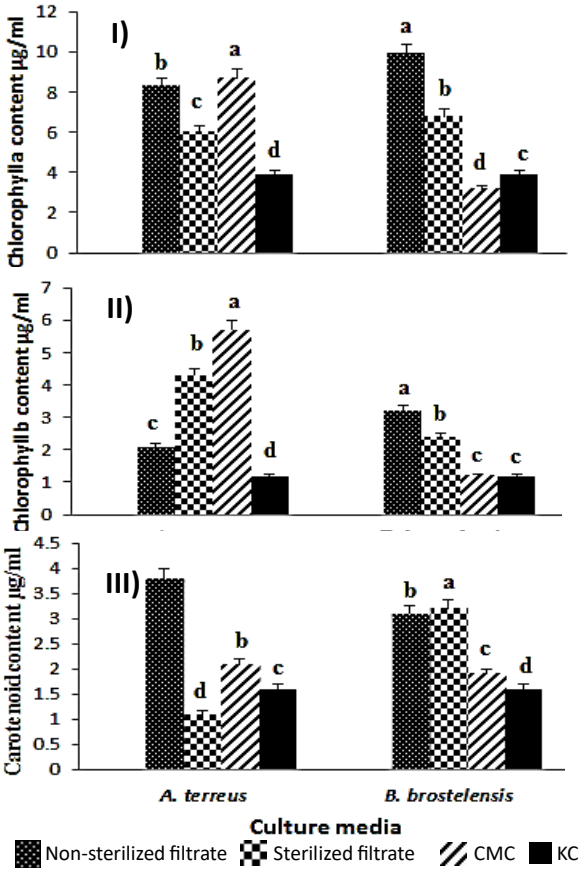


Figure 6. Pigment contents in *C. vulgaris* grown on rice filtrates of *A. terreus* and *B. borstelensis* separately (I) Chlorophyll *a*, (II) Chlorophyll *b*, (III) Carotenoid. Different letters on the same column for each parameter differ significantly at p≤ 0.05.

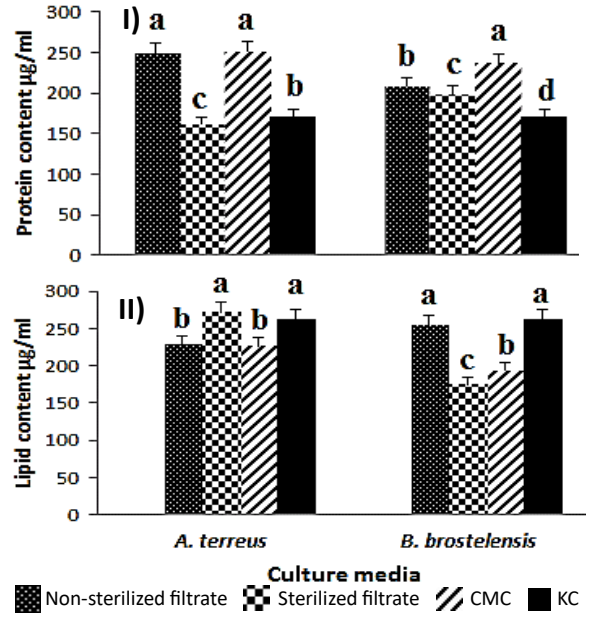


Figure 7. I) Protein content (µg/ml), II) Lipid content (µg/ml), of *C. vulgaris* grown on rice filtrate of *A. terreus, B. borstelensis.*

total composition of the microalgae, located between the spaces of the thylakoids in the chloroplasts. Similarly, Liang *et al*. (2009) reported lower lipid content in the autotrophic growth of *C. vulgaris* compared with mixotrophic conditions. On the other hand, fan et al. (2015) reported that the highest lipid content was observed in autotrophic cultures of *C. pyrenoidosa*. This demonstrates that lipid accumulation by algal cells is influenced by algal species and growth conditions. The composition of the medium will affect the biomass and lipid content that will be produced by microalgae.

Those results are consistent with those of Liu *et al*. (2009) who found lower number of carotenoids in mixotrophic cells when compared to cells grown on photoautotrophic culture. This coincided with the results of Kong *et al*. (2012) who reported the same trend for *C. vulgaris* under mixotrophic conditions. Xie *et al*. (2017) confirmed present stimulated protein synthesis in *C. vulgaris* on an inorganic (nitrate) nitrogen rich medium. Mohammad *et al*. (2023) reported that, protein in autotrophic cultivation were slightly higher than that produced under mixotrophic conditions with non-significant differences.

CONCLUSIONS

Biocleaning of natural cellulosic wastes by combining cellulosic digested microbes with mixotrophic nutrition microalgae is the best way to design a microbial biorefinery. The present study describes the isolation and identification of two digested cellulosic microorganisms, *Aspergillus terreus* and *Brevibacillus borstelensis*. Obtained results showed the highest recorded sugar yield through cellulosic biodegradtion of 648.1 µg/ml on the 8th day of incubation with *A. terreus* (µg/ml untreated straw), and 814 µg/ml on the 4th day with *B. borstelensis* (µg/ml treated straw). Mixotrophic nutrition of *Chlorella vulgaris* on decomposing rice husk filters led to enhancing of algal growth, pigments, protein and lipid contents. Cellulose pollution has been overcome and converted into viable energy. Promoting biomass production has confirmed the existence of a safe and viable system for converting cellulosic waste into a useful product.

CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest.

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There is no fund

AUTHOR CONTRIBUTIONS

Eithar El-Mohsnawy contributed to the study conception and experimental designs. Material preparation, sample assembly, data collection and analysis were performed by Azza El-Naggar, Eithar El-Mohsnawy and Yasser El-Halmouch. The first draft of the manuscript was written by Azza El-Naggar and Eithar El-Mohsnawy. Statistical analysis and Figures preparation was performed by all authors. All authors commented on the 1st version of the manuscript. All authors read and approved the final manuscript.

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