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Research article

Rice straw as microalgal biofilm bio-carrier: Effects of indigenous microorganisms on rice straw and microalgal biomass production

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ABSTRACT

Microalgal biofilm cultivation is a promising method for efficient microalgae production. However, expensive, difficult-to-obtain and non-durable carriers hinder its up-scaling. This study adopted both sterilized and unsterilized rice straw (RS) as a carrier for the development of microalgal biofilm, with polymethyl methacrylate as control. The biomass production and chemical composition of *Chlorella sorokiniana*, as well as the microbial community composition during cultivation were examined. The physicochemical properties of RS before and after utilized as carrier were investigated. The biomass productivity of unsterilized RS biofilm exceeded that of suspended culture by 4.85 g $m^{-2}{\cdot}d^{-1}$. The indigenous microorganisms, mainly fungus, could effectively fixed microalgae to the bio-carrier and enhance its biomass production. They could also degrade RS into dissolved matters for microalgal utilization, leading to the physicochemical properties change of RS in the direction which favored its energy conversion. This study showed that RS can be used effectively as a microalgal biofilm carrier, thus presenting a new possibility for the recycling of rice straw.

1. Introduction

The globe is experiencing an energy supply problem, and microalgae are gaining popularity as a possible source of biodiesel (Chang et al., 2023; Patwardhan et al., 2022). Currently, large-scale commercial microalgae culture is primarily based on suspended culture, which results in the problems of separating microalgae from their growth media due to the minute size of microalgae cells and the electrostatic repulsion between them (Singh et al., 2021). Although traditional physical harvesting methods such as centrifugation, filtration, and air flotation can achieve high harvesting rates, they incur high harvesting expenses, which typically contribute to 20–30% of total production costs (Chang et al., 2022; Zhu et al., 2020). Chemical methods, such as chemical flocculation, can harvest microalgae quickly, while they may damage microalgae quality and cause environmental pollution (Rwehumbiza et al., 2012). Numerous recent researches have demonstrated that microalgal biofilm technology has promising application potential for microalgae production. The attached microalgal biomass can be harvested in a straightforward method (e.g., mechanical scraping) with equivalent harvesting efficiency to centrifugation in a suspended system, but with a 99.7% reduction in energy requirements (Huang et al., 2016). Furthermore, biofilm has a better light penetration efficiency and mass transfer rate, promoting microalgal growth (Li et al., 2022; Zhang et al., 2018). But even so, microalgal biofilms face the issue of carrier selection as well. Common carriers such as glass fiber and metal materials are expensive, and others like cotton, polystyrene foam, or cardboard are mechanically weak and un-durable, requiring additional processing to increase the roughness of their surface for microalgal attachment (Li et al., 2022; Zhuang et al., 2018). Therefore, there is still a requirement to develop economical and efficient biofilm carriers for the attached cultivation of microalgae.

Rice straw (RS) is the most prevalent agricultural residue, with an estimated annual global production of about 740.95–114.2 million tons (Moliner et al., 2016). Various lignocellulosic materials such as pine

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sawdust and rice husk have been successfully employed as biofilm carriers in microalgal cultivation (Rawindran et al., 2022; Zhang et al., 2019). RS shares similar physicochemical properties with rice husk and has a rough surface suitable for microalgal attachment, and microalgae usually attach to its surface in the rice fields. Thus, as a low-cost and easily available lignocellulosic resource, RS has enormous potential as a microalgal biofilm carrier.

In addition, RS is also a promising feedstock for biodiesel production, but its crystalline cellulose and complex composition of RS make its utilization challenging. Therefore, proper pretreatment is necessary to reduce cellulose polymerization and crystallinity index and enhance accessibility (Ma et al., 2023; Uthandi et al., 2022). Among the available pretreatment options, biological pretreatment is preferable to the energy-intensive physical and the hazardous chemical methods. The pretreatment of RS with screened microorganisms has been intensively studied (Kumar et al., 2015). However, it is noteworthy that unsterilized RS has microorganisms on its surface naturally. Treatment of RS with indigenous microorganisms eliminates the need for tedious screening of target microorganisms and saves energy consumption for sterilization. And numerous studies have demonstrated that microbes and microalgae may create mutually beneficial symbiotic interactions to boost each other's growth (Chu et al., 2021; Li et al., 2022). Employing RS as a biofilm carrier for microalgae production can be advantageous, as it facilitates the accumulation and harvesting of microalgal biomass, and can also be pretreated by microorganisms.

To date, no study has verified the feasibility of RS as a microalgal biofilm carrier, and neither have we investigated the changes in its physicochemical properties after being utilized as a carrier. In this work, the biomass productivity and chemical composition of *Chlorella sorokiniana*, as well as the microbial community composition during cultivation were examined to verify the feasibility of RS as a microalgal biofilm carrier. Moreover, the bioleaching effects on RS were examined using fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and thermogravimetric analysis (TG) during the cultivation of biofilm. These findings can serve as a theoretical foundation for the expansion of microalgal biofilm technology and the exploitation of RS resources.

2. Materials and methods

2.1. Rice straw and microalgal strain

RS was collected from Jiulong village in Suizhou city, Hubei province, China. Following a period of exposure to sunlight of 15 days, it was pulverized and screened into particles between 380 and 830 μm in size. Sample was oven dried at 50 °C for another 3 days for further experiment.

The *Chlorella sorokiniana* FACHB-24 was procured from the Institute of Hydrobiology, Chinese Academy of Science, PR China, and kept in BG11 medium under controlled conditions of temperature (26 ± 1 °C) and illumination (45 ± 2 μmol $m^{-2}·s^{-1}$).

2.2. Microalgal biofilm bioreactor

This study used a pre-developed photobioreactor, which consists of four channels, a four-channel peristaltic pump, and four medium reservoirs. The light receiving area of each channel was 0.015 m^2. The details of the photobioreactor are described in Fig. S1.

2.3. Experimental design

2.3.1. Rice straw as microalgal biofilm carrier

In channel 1 of the bioreactor, 3.5 ± 0.5 g of unsterilized RS was spread evenly (RS-R), while an equivalent amount of sterilized RS was distributed evenly in channel 2 (RS-S). Channel 3 only used the material of bioreactor, polymethyl methacrylate, as the biofilm carrier (PMMA), while channel 4 was filled with the same amount of unsterilized RS without microalgae inoculation (RS-W). For the suspended culture (SUS), a 5 L flask was covered with aluminum foil, with 0.015 m^2 of its bottom exposed to the same light source as the reactor channel. 2 L of sterilized BG11 medium was added to each medium reservoir or flask, and 5 mL of *Chlorella sorokiniana* solution (with an optical density at 685 nm of 0.356) during the logarithmic phase was added to all of them, except for channel 4. The medium in the reservoirs was then cycled using a four-channel peristaltic pump. The flow rate of each channel was set to 40 ± 5 mL min^{-1}, the temperature was kept at 32 ± 2 °C, the photoperiod was 14 h/10 h (light intensity was 120 ± 10 μmol $m^{-2}·s^{-1}$). The experiment was conducted for 10 days in triplicates. After cultivation, the microalgal biomass from each treatment was harvested for further analysis.

2.3.2. Bioleaching of rice straw

A bioleaching experiment was conducted to assess the effects of using RS as a carrier on its properties. 3.5 ± 0.5 g of unsterilized RS was evenly spread in each 4 channels of the reactor and 2 L of sterilized deionized water was added to each medium reservoir. After that, peristaltic pumps were started to circulate the liquid and the system operating conditions were the same as in Section 2.3.1. It is worth noting that no nutrients were added to the leachate reservoirs and no microalgae were inoculated. Deionized water served only as a moisture supplement to wet the RS samples and replenish evaporated water. The leachate from each reservoir was sampled and analyzed at days 1, 5, 10, 15, and 20. Each of the four channels was run for a specific period, i.e., 5, 10, 15, and 20 days, and the materials collected for further analysis. Also, 250 mL of leachate from each reservoir was collected and sterilized. The sterilized leachate was used as culture medium for the growth of *Chlorella sorokiniana*, with sterilized BG 11 medium serving as the control. The culture was conducted in a flask (1 L) and lasted for 7 days with a 1 L min^{-1} aeration speed of 2% CO_2, and the light and temperature conditions were consistent with Section 2.3.1. The cell density was tested through hemacytometer counted under optical microscope (CKX41, OLYMPUS, Japan) and expressed as cells·mL^{-1} every 3–24 h.

2.4. Analytic methods

2.4.1. Microalgal biomass production and composition analysis

The biofilm mixtures were obtained by scraping. In order to separate the carrier material and microalgae cells, the mixture was combined with 200 mL deionized water and stirred for 10 min at a speed of 2000 rpm min^{-1} (JJ-1, HUAOU, China), then ultrasonically treated in an ultrasonic bath (KQ-100DB, Kunshan, China) for 40 min (40 kHz, 100 W). After filtering via filter paper, the suspended cells were harvested by centrifugation at 7000 rpm (H2050R, CENCE, China) for 10 min, rinsed with deionized water three times and then recovered by centrifugation. The residuals were subjected to freeze-drying (FD5-3, GOLD-SIM, USA) for yield and composition analysis. Besides, the *Chlorella sorokiniana* cultured in BG11 were harvested by direct centrifugation and rinsed in the same way of biofilm and freeze-dried (FD5-3, GOLD-SIM, USA).

For composition analysis, all microalgal biomass samples were ground and sieved to obtain a particle size below 74 μm. The lipid, protein, carbohydrate, and ash contents were determined using the method described by Zhang et al. (2018). The fatty acid methyl ester (FAME) profiles was determined using the method described by Zhang et al. (2018). The biodiesel properties were calculated according to other studies and the results were shown in Table S1 (Nascimento et al., 2013). An elemental analyzer (Vario Micro cube, Elementar, Germany) was used for the elementary analysis.

2.4.2. Microbial community analysis

In order to analyze the fungal community during the culture process, several small pieces of mycelium-filled materials from channel 1 and channel 4 were randomly selected on day 7 and stored at −80 °C for

further analysis. The samples were dispatched to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) to undergo Illumina high-throughput sequencing assay. The metagenomic DNA of the samples was extracted using the E. Z.N.A. Soil DNA Kit (Omega, USA) according to the user instructions. The V5–V7 region of the 18 S rDNA genes was amplified by the universal primers SSU0817F (5′-TTAGCATGGAATAATRRAATAGGA-3′) and 1196 R (5′-TCTGGACCTGGTGAGTTTCC-3′). Sequencing of the PCR products was conducted on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.), and the bioinformatics analysis was then performed on Majorbio I-Sanger (https://cloud.majorbio.com/).

2.4.3. Analysis of the properties of bioleached rice straw and leachate

The collected RS after the experiment of Section 2.3.2 was initially washed trice with deionized water and then dried at 50 °C for 3 days. Mass loss rate of RS during the bioleaching process was calculated as follow:

$$\alpha_t = (m_0 - m_t)/m_0 * 100\% \quad (1)$$

Where α_t is the mass loss rate of RS after t days' bioleaching by indigenous microorganisms, m_0 is the material weight before bioleaching process, m_t is the material weight after t days' bioleaching.

Bioleached RS and raw RS material were ground and sieved to gain a particle size below 74 μm. The samples were then analyzed by an elemental analyzer (Vario Micro cube, Elementar, Germany) and a proximate analyzer (TGA 2000, Las Navas, Spain) respectively. The higher heating value (HHV) was calculated according to the method reported elsewhere (Moliner et al., 2016). The cellulose, hemicellulose and lignin contents of the samples were measured in accordance with the methods reported by Li et al. (2022). Mass loss rates of hemicellulose, lignin, cellulose and ash in the samples were calculated according to Eq. (2):

$$\alpha_{i,t} = \left(m_0 * c_{i,0} - m_t * c_{i,t}\right)/\left(m_0 * c_{i,0}\right) * 100\% \quad (2)$$

Where $\alpha_{i,t}$ is the mass loss rate of the component i (hemicellulose, lignin, cellulose and ash) after t days' bioleaching, $c_{i,0}$ is the content of the component i in the raw RS, $c_{i,t}$ is the content of the component i in the t days' bioleached RS.

The FTIR spectrum of the samples in KBr pellets (1:100, wt/wt) was obtained by a FTIR spectroscopy (VERTEX 70, Bruker, Germany) with a resolution of 4 cm^{-1} and 32 scans between 4000 and 400 cm^{-1} under 26 °C. Crystallinity of the samples were determined through an X-ray diffractometer (X'Pert PRO, PANalytical B.V., Holland). Thermogravimetric analysis was performed using a simultaneous Thermogravimetry/Differential Thermal Analysis (TG/DTA) analyzer (Diamond TG/DTA, PerkinElmer, USA). The sampled leachate was filtered through a 0.45 μm filter and then the concentrations of N–NO_3^-, N–NH_4^+, TN (Total Nitrogen), TP (Total Phosphorus) and COD (Chemical Oxygen Demand) were measured according to ASTM standards (ASTM D6508 for N–NO_3^-, ASTM D6919 for N–NH_4^+, ASTM D8001 for TN and TP, ASTM D1252 for COD).

2.4.4. Analysis of environmental scanning electron microscope (ESEM)

During the microalgal biofilm cultivation process, two small pieces of RS samples were collected for morphological analysis using an environmental scanning electron microscope (QUANTA 200, FEI, Holland) on day 8. This was repeated on days 0, 1, 5, 10, 15, and 20 of the bioleaching process. Before analysis, one piece of material from each channel was first washed for three times using deionized water. Following that, the washed, un-washed, and raw RS samples were fixed with 2.5% glutaraldehyde for 4 h and then rinsed trice using a phosphate buffer solution (comprised of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, with a pH 7.4). After fixation, the fixed samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 85% and 100%, v/v) for 15 min each. Isoamyl acetate was added twice to replace the ethanol inside dehydrated samples within 20 min, and they were then frozen at −20 °C, −40 °C, −80 °C for 4 h each. Finally, the samples were freeze-dried for 12 h and sputter-coated with a thin gold layer for surface morphology analysis.

2.5. Statistical analysis

All the analytic experiments were replicated thrice and average values were reported. Results were performed with EXCEL 2013 (Microsoft Office Enterprise, USA), OriginPro 9.0 (OriginLab, USA) and GraphPad Prism 9 (GrahpPad Software, USA). Analysis of Variance (ANOVA) was applied wherever applicable.

3. Results and discussion

3.1. Rice straw as microalgal biofilm carrier

3.1.1. Biomass production

Table 1 shows the average biomass productivity of the four different treatments during the 10-day culture, where the biomass productivity of the suspended culture was measured based on the area exposed to light. The results showed that the biomass productivity of the four treatments was ranked as follows: RS-R (11.18 ± 0.23 g m^{-2}·d^{-1}) > RS-S (8.79 ± 0.42 g m^{-2}·d^{-1}) > SUS (6.33 ± 0.41 g m^{-2}·d^{-1}) > PMMA (3.46 ± 0.22 g m^{-2}·d^{-1}). The biomass productions of *Chlorella sorokiniana* using RS as carrier were significantly higher than that on PMMA and suspended culture ($P < 0.05$), indicating the feasibility of using RS as microalgal biofilm carrier. RS has a natural rough surface (Fig. S2), Zhang et al. (2020) found that the hydrodynamic free zone formed on the rough surface promotes the retention of microalgae cells, so that they can be well deposited on the surface of the carrier, thereby promoting the generation of microalgal biofilms. The effective light penetration and high mass transfer rate of biofilm contributed to the superior growth performance of microalgae refer to the suspended culture (Li et al., 2022; Wang et al., 2015). In contrast, PMMA material had a relatively

Table 1
Summary of the productivity and FAMEs profile for the microalgal biomass (means ± sd).

Parameters		RS-R	RS-S	PMMA	SUS
Biomass productivity (g·m^{-2}·d^{-1})		11.18 ± 0.23	8.79 ± 0.42	3.46 ± 0.22	6.33 ± 0.41
Saturated fatty acids (%)	C16:0	25.99 ± 0.24	26.24 ± 0.61	25.43 ± 1.78	26.59 ± 0.14
	C18:0	0.52 ± 0.74	0.61 ± 0.87	1.37 ± 0.05	1.36 ± 0.02
	Subtotal	26.51 ± 0.99	26.85 ± 1.47	26.81 ± 1.73	27.95 ± 0.12
Monounsaturated fatty acids (%)	C16:1	7.34 ± 0.29	7.76 ± 0.90	6.70 ± 0.55	6.26 ± 0.07
	C18:1	28.60 ± 0.10	27.34 ± 1.88	19.08 ± 8.00	13.35 ± 0.10
	Subtotal	35.94 ± 0.19	35.11 ± 0.99	25.78 ± 8.55	19.61 ± 0.17
Polyunsaturated fatty acids (%)	C16:2	3.50 ± 0.17	3.18 ± 0.29	4.01 ± 0.50	3.67 ± 0.02
	C18:2	17.79 ± 1.01	18.05 ± 0.64	24.35 ± 5.22	28.12 ± 0.11
	C16:3	4.40 ± 0.17	3.88 ± 0.90	12.42 ± 6.43	17.04 ± 0.12
	Subtotal	25.70 ± 1.01	25.11 ± 1.84	40.78 ± 11.15	48.83 ± 0.24
Others (%)		11.85 ± 0.17	12.93 ± 1.35	6.63 ± 4.33	3.61 ± 0.05
C16–C18 (%)		88.15 ± 0.17	87.07 ± 1.35	93.37 ± 4.33	96.39 ± 0.05
Total (% of dw)		1.13 ± 0.03	1.05 ± 0.08	1.28 ± 0.13	1.08 ± 0.16
Biodiesel productivity (mg·m^{-2}·d^{-1})		126.33 ± 3.35	92.30 ± 7.03	44.29 ± 4.50	68.36 ± 10.13

smooth surface, making it challenging for *Chlorella sorokiniana* to adhere to form a stable biofilm (Li et al., 2022). Most *Chlorella sorokiniana* cells were washed into the culture medium by water during the culture process, so that PMMA biofilm obtained the lowest biomass. This is evident from Fig. S3, which shows robust proliferation of indigenous microorganisms, mainly fungi, carried by unsterilized RS during the culture process.

Previous studies have suggested that co-culturing microalgae and fungi can establish synergistic metabolism, leading to improved growth for both organisms. Fungi produce extracellular enzymes that transform solid organic matter into soluble substances that can be used by microalgae. Furthermore, carbon dioxide produced by fungal respiration can also be utilized by microalgae, while fungi can immobilize microalgae cells, promoting the aggregation of microalgae cells through hydrophobic and electrostatic interactions and bio-flocculation mediated by specific components on the cell wall (Yang et al., 2019; Zheng et al., 2019). Thus, co-cultivation with fungi has become a widely used technique for harvesting microalgae during large-scale cultivation (Chu et al., 2021). However, few studies have combined fungi with microalgal biofilms. The combination of these characteristics of fungi with microalgal biofilms can form biofilms with stronger mechanical stability, thereby increasing the yield of biofilms, which may be another reason for the highest biomass of *Chlorella sorokiniana* biofilm on unsterilized RS. ESEM results also showed the complicated relationship between fungi and microalgae (Fig. S4). The microalgae cells aggregated and adhered to the fungal hyphae embedded in the carrier, improving biofilm adhesion, thus increasing the microalgal biomass production. Moreover, the *Chlorella sorokiniana* biofilm cultured on RS was easy to harvest. After culture, RS couple with the microalgae could be rolled up like a blanket (Fig. S3). Such mixed biomass could be used as nutrient-rich livestock and poultry feed. Besides, this biofilm, which was strongly bonded to the carrier, was highly resistant to shear losses and could withstand high shear stresses during the wastewater treatment process.

3.1.2. Chemical composition

Fig. 1a and b describes the lipid, protein, ash and carbohydrate contents and productivity of microalgal biomass from biofilm and suspended cultures. PMMA biofilm and suspended *Chlorella sorokiniana* had higher lipid and protein contents than RS-R and RS-S biofilm. Zhuang et al. (2018) found there was no significant relationship between culture methods (attached or suspended) and microalgal composition. Cell components (lipids, proteins, carbohydrates, etc.) major vary with the environment and culture conditions (Wu et al., 2023a). Some substances of straw would be dissolved into the culture medium during the culture process, and indigenous microorganisms might also decompose RS into small molecules and entered into the liquid environment, thus changing the composition of the culture medium to a certain extent. This might be the reason why the chemical composition of RS biofilm is quite different from that of PMMA biofilm and suspended culture. Moreover, biofilm on RS was thicker than the other treatments, the light absorption and scattering by pigments and cells, respectively, led to a quick decrease in light intensity (Ma et al., 2022; Ye et al., 2023). As a result, the microalgal cells located in the inner layer of the biofilm received less light. Studies have shown that light starvation can affect the synthesis of lipids in microalgae cells, resulting in low lipid content in the biofilm (Ma et al., 2023). In addition, the interaction between fungi and microalgae as well as the chemical composition of fungi could affect the chemical composition of biofilm on RS-R. The protein content (37.84 ± 0.05%) of microalgal biomass cultured using unsterilized RS as carrier was higher than that of RS-S (32.82 ± 0.05%), and its ash content (11.37 ± 0.08%) was lower than that of RS-S (16.66 ± 0.54%), indicating that the *Chlorella sorokiniana* produced on RS-R was more suitable for feed and biological fertilizer than that of RS-W. Meanwhile, the productivities of protein, lipid and carbohydrate of the biofilm on RS-R were 4.23 ± 0.00, 1.00 ± 0.09 and 4.81 ± 0.06 g m^{-2}·d^{-1}, respectively. These values were significantly higher than that of PMMA and suspended culture ($P < 0.05$), indicating that RS as microalgal biofilm carrier also has a greater advantage in value-added products production.

Microalgae can accumulate almost 70% of its dry weight of lipids in cell under the ideal circumstances (Rosmahadi et al., 2021). The fuel qualities of biodiesel are highly connected to lipid components (fatty acids) in microalgae. *Chlorella sorokiniana* has relatively high contents of short-chain fatty acids, especially C16–C18 fatty acids, which are appropriate for the manufacture of biodiesel. Table 1 shows the lipid profile of *Chlorella sorokiniana* collected from biofilm and suspended culture. Their saturated fatty acids were comparable, ranging around 26%, while the levels of monounsaturated fatty acids and polyunsaturated fatty acids were significantly different. The DU (degree of unsaturation) of fatty acids in PMMA and SUS (107.34% and 117.27%) were higher than that of RS-R and RS-S (87.34% and 85.33%). A higher degree of fatty acid unsaturation is beneficial for low-temperature performance and kinematic viscosity of the produced fuel. Despite this, higher unsaturation levels might also make biodiesel more susceptible to oxidative deterioration (Menegazzo and Fonseca, 2019). In addition, the biofilm on RS-R had 11.85% of other fatty acids (C14:0, C20:5, etc.), suggesting that the percentage of C16–C18 fatty acids was 88.15%. This value was lower than that of PMMA (93.37%) and suspended culture (96.39%).

3.1.3. The composition of microbial community

As shown in Table 2, the coverage index of the two samples collected from the biofilm of RS-R and RS-W reached 0.99, indicating that the sequencing result could truly reflect the diversity of the microbial community. The two samples shared 12 operational taxonomic units (OTUs) with each other and had 14 (RS-R) and 15 (RS-W) OTUs,

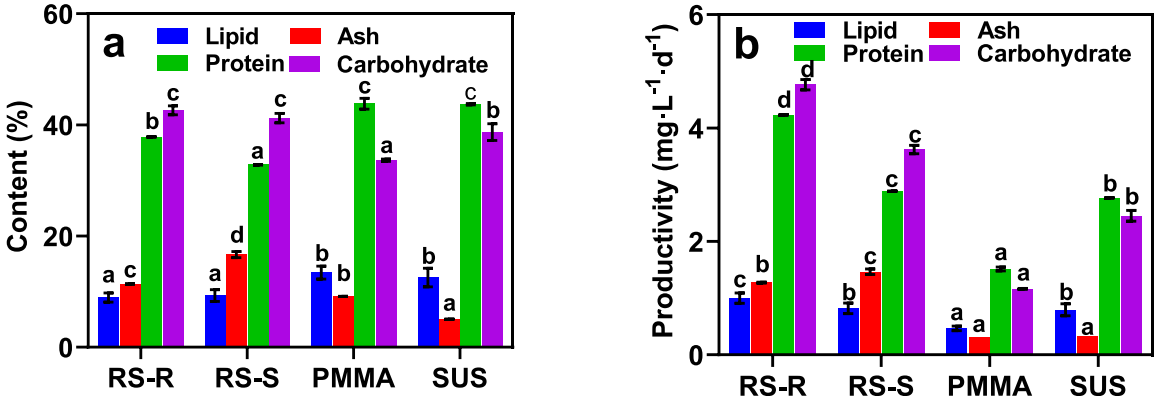

Fig. 1. The lipid, protein, ash and carbohydrate contents (a) and productivity (b) of microalgal biomass from biofilm and suspended cultures.

Table 2
Microbial community diversity index at OTU level.

Group	Sobs	Shannon	Simpson	Ace	Chao	Coverage
RS-R	14	0.24	0.92	14.83	14	0.99
RS-W	15	0.47	0.79	15.37	15	0.99

respectively (Fig. 2a). Additionally, the Chao and ACE estimators (community richness index), and Shannon and Simpson indexes (community diversity index) suggested that the microbial richness and diversity of RS-W were slightly higher than in RS-R (Table 2). These results demonstrated the microbial community composition on unsterilized RS remain similar whether or not microalgae were present. The circos diagram (Fig. 2b) displays the detailed community microbes at the order level. *Sordariales* was the dominant order in both samples, with percentages of 95.62% in RS-R and 88.46% in RS-W. *Sordariales*, a large group of fungi commonly involved in plant material decay, includes *Chaetomium*, an important and well-known species that decomposes wood and plant materials (Li et al., 2022). Li et al. (2003) found that *Chaetomium thermophilum* CT2 can produce endocellulase to degrade cellulose. Polysaccharide mono-addition oxidases (PMOs) commonly act in concert with cellulose disaccharide dehydrogenase and other cellulolytic enzymes to cleave cellulose molecules internally in an oxidative manner, and 30–44 genes encoding PMOs are exist in *Chaetomium* (Žifčáková and Baldrian, 2012). In addition, *Chaetomium* sp. CQ31 can produce xylanase to degrade hemicellulose, while *Chaetomium globosum* can secrete laccase to degrade lignin (Dou et al., 2022; Liu et al., 2022).

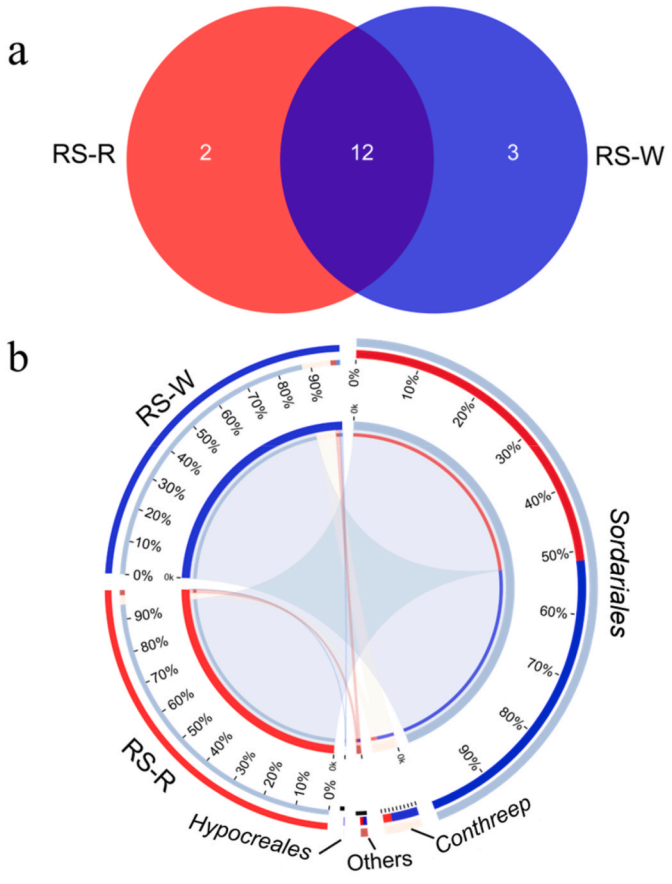

Fig. 2. Venn diagram of microbial community in the samples from RS-R and RS-W (a), Circos diagram of the distribution of microbial community in the samples from RS-R and RS-W at order level (b).

3.2. Effect of bioleaching on RS composition and properties

3.2.1. Chemical composition and morphology of the feedstock

To investigate the effect of indigenous microorganisms on RS and the changes in its physicochemical properties during biofilm cultivation, a bioleaching experiment on RS was conducted. Table S1 shows that the mass loss of RS during bioleaching increased significantly with the increase of treatment time ($P < 0.05$). After 20 days of bioleaching, the weight of RS decreased by approximately 49.03%. For the initial five-day period, hemicellulose degradation accounted for 64.99%, lignin degradation 8.66%, and cellulose degradation only 1.29%. The extensive degradation of hemicellulose in the first five days destroyed the protection mechanism of cellulose and increased the accessible area of enzyme, thereby promoting the subsequent biodegradation of cellulose. After the fifth day, hemicellulose and lignin were basically not lost, while the mass loss rate of cellulose increased from 1.29% to 39.33% within 5–20 days.

The proximate analysis results (Table S1) showed that the ash content of RS decreased sharply from 12.09% to 5.42% ($P < 0.05$) after 20 days of bioleaching, while the volatile increased significantly from 65.81% to 73.48% ($P < 0.05$). X-ray Fluorescence (XRF) analysis indicated that the decrease of ash content was mainly resulted from the leaching out of Si, K, Mg and S from the feedstock. Besides, XRF analysis illustrated that Si was the most abundant inorganic elements in RS feedstock, but it was significantly decreased from day 5 to day 20 ($P < 0.05$). This decrease illustrated that ash continued to leach even through its content remained unchanged. The ultimate analysis showed an increase in the content of carbon (C) and hydrogen (H) while the content of oxygen (O) reduced, leading to an increase in the higher heat value (HHV) of RS materials. Nitrogen (N)content is typically connected with microorganisms, and a progressive increase in N concentration suggested ongoing growth of indigenous microorganisms on RS (Fig S3).

In summary, the leaching process significantly altered the composition and properties of RS, stimulated by the collaboration of indigenous microorganisms and water flow. Water can dissolve part of cellulose and hemicellulose and dissolve most of the inorganic salts. Microorganisms initially decompose hemicellulose to expose cellulose to promote enzyme accessibility, and then most of the cellulose is biodegradable in the later period. The RS degradation could also be observed according to the ESEM images (Fig. S2), especially for the sample collected at day 15, in which cellulosic fiber had been significantly degraded. It is also observed that the RS surface became rough as the bioleaching time increased, e.g., surface of the sample at day 20 was the roughest as compared with others. The degradation of RS resulted in an abundance of pores and specific surface area, facilitating microalgae cell attachment and energy conversion of RS.

3.2.2. Spectrum and thermogravimetric analysis

Fig. 3a shows the FTIR spectra of raw RS and bioleached RS. Compared to the raw RS, the FTIR spectra of the bioleached RS show a decrease in both the smaller shoulder peak at 1730 cm^{-1} and the peak at 1650 cm^{-1} (attributed to the bending pattern of the absorbed water), indicating degradation of hemicellulose (Liu et al., 2016; Wu et al., 2023b). The 1320 cm^{-1} (C–O vibration in the syringyl ring) and 1375 cm^{-1} (beta ether bond) absorption peaks are characteristic peaks of lignin (Liu et al., 2016). The attenuation of these two groups in the leached RS spectra implies degradation of the lignin. Decrease in the intensities of the 1105 cm^{-1} and 899 cm^{-1} peaks showed the degradation of both crystalline cellulose and hemicellulose (Liang et al., 2014).

XRD patterns demonstrated that bioleached RS samples showed similar X-ray diffraction spectrum to the raw sample (Fig. 3b). The crystallinity index (CrI) of bioleached RS was first increased from 50.03% to 58.82% after 5 days bioleaching, and then reduced gradually to 48.71%. This indicated that the crystalline cellulose content in the RS was first increased and then decreased during the leaching. It is consistent with the conclusion reported by Liang et al. (2014). The

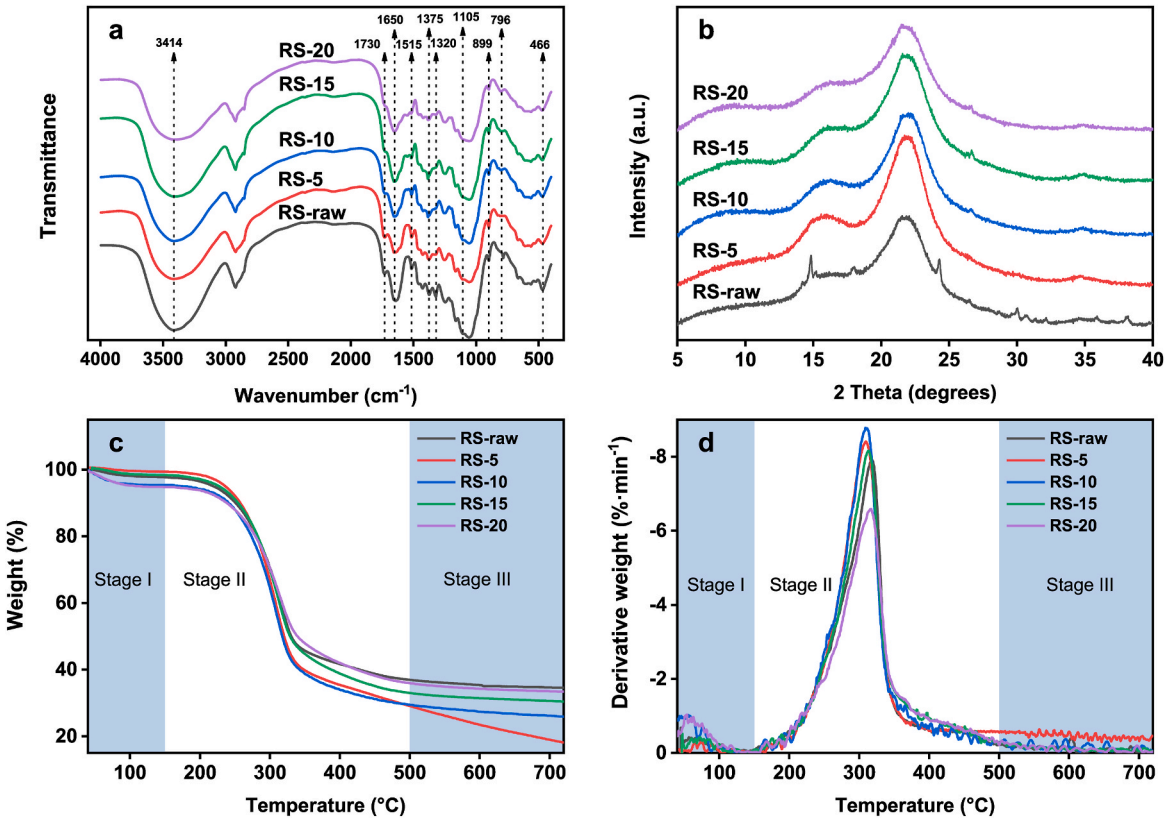

Fig. 3. FTIR spectrograms (a), XRD patterns (b), thermogravimetric profiles (c) and differential thermogravimetric profiles (d) of the raw rice straw (RS-raw), 5 days' bioleached rice straw (RS-5), 10 days' bioleached rice straw (RS-10), 15 days' bioleached rice straw (RS-15) and 20 days' bioleached rice straw (RS-20).

reduction of crystallization index will facilitate the conversion of RS to biofuel (Uthandi et al., 2022).

Fig. 3c and d shows the thermogravimetric (TG) and differential thermogravimetric (DTG) curves of the raw RS and bioleached RS. Decomposition data (Table S2) derived from the TG and DTG profiles demonstrate the thermo-chemical change of RS after bioleaching. In general, Larger T_{50} values indicate higher lignin levels in the sample because lignin has higher thermal stability than hemicellulose and cellulose (Liu et al., 2016). Meanwhile, R_{50} mainly refers to the specific surface area and larger value of R_{50} means relatively larger specific surface area of the sample because structure with abundant pores generally promote the pyrolysis process (Liu et al., 2016). After 10 days of bioleaching, T_{max} decreased from 331 °C to 317 °C and R_{max} increased from 7.93%·min^{-1} to 8.78%·min^{-1}, T_{50} decreased from 318 °C to 309 °C and R_{50} increased from 4.33%·min^{-1} to 8.17%·min^{-1}. The increased T_{50} and T_{max} as well as the decreased R_{50} and R_{max} after day 10 indicated increased thermal stability of the 15 days and 20 days samples compared with the 5 days and 10 days samples.

FTIR and composition analysis results indicated the cellulose have been degraded after leaching, and the first increasing and then decreasing of crystallinity index of the sample also indicated the sharp degradation of cellulose. The degradation of cellulose after day 10 led to relatively increase in lignin content of RS, resulting in a change in its thermal stability. Thermogravimetric analysis demonstrated that the samples after 5 or10 days' bioleaching were thermally unstable and more susceptible to pyrolysis. Meanwhile, the RS pretreated for 5 or 10 days had a higher cellulose content and lower ash and hemicellulose content, resulting in a more accessible biodegradable process after day 10 due to significant cellulose degradation. Thus, the optimal bioleaching time for promoting energy conversion of RS would be 5–10 days.

3.3. Characterization of the leachate from the bioleaching process

During the bioleaching of RS, the leachate from the system was monitored. Fig. 4g demonstrates the gradually changed color of collected leachate from different treatments. Regarding the concentrations of nutrients, it varied similarly among the four treatments. Fig. 4 shows that nutrients concentrations were dramatically increased in the first day and reached peak values, with N–NH_4^+, N–NO_3^-, TN, TP and COD up to 1 mg L^{-1}, 6 mg L^{-1}, 9 mg L^{-1}, 3 mg L^{-1} and 110 mg L^{-1} respectively. After day 1, the N–NO_3^-, TN and TP concentrations were decreased significantly with the process continued. N–NH_4^+ concentrations were decreased firstly followed by an increase later. Similarly, the COD values decreased within the initial 5 days but slightly increased afterward. In this study, no additional chemicals were added to the reservoir. Therefore, the pollutants were all produced by bioleaching processes. The subsequent decrease in pollutant concentrations may be mainly attributed to the apparent proliferation of associated indigenous microorganisms, which consume certain amounts of nitrogen, phosphorus and COD as nutrients.

Therefore, in order to verify that indigenous microorganisms have degraded the RS and promoted the growth of microalgae, the leachates produced by bioleaching were used as medium to culture *Chlorella sorokiniana*. Fig. 4f showed that no significantly growth inhibition was observed when using leachate for microalgal cultivation within the first 5 days, indicating *Chlorella sorokiniana* could grow robustly with the leachate from different treatments. But to a certain extent, the microalgae grew better in the tested leachate compared with BG 11 within the first 5 days, especially with the leachate from 5 days to 15 days treatments. This could be mainly due to the organic matters (COD, all larger than 60 mg L^{-1}) contained within the leachate that served as organic carbon source for microalgal cultivation and promoted its growth (Zhang and Hu, 2012). The high COD concentration might be mainly

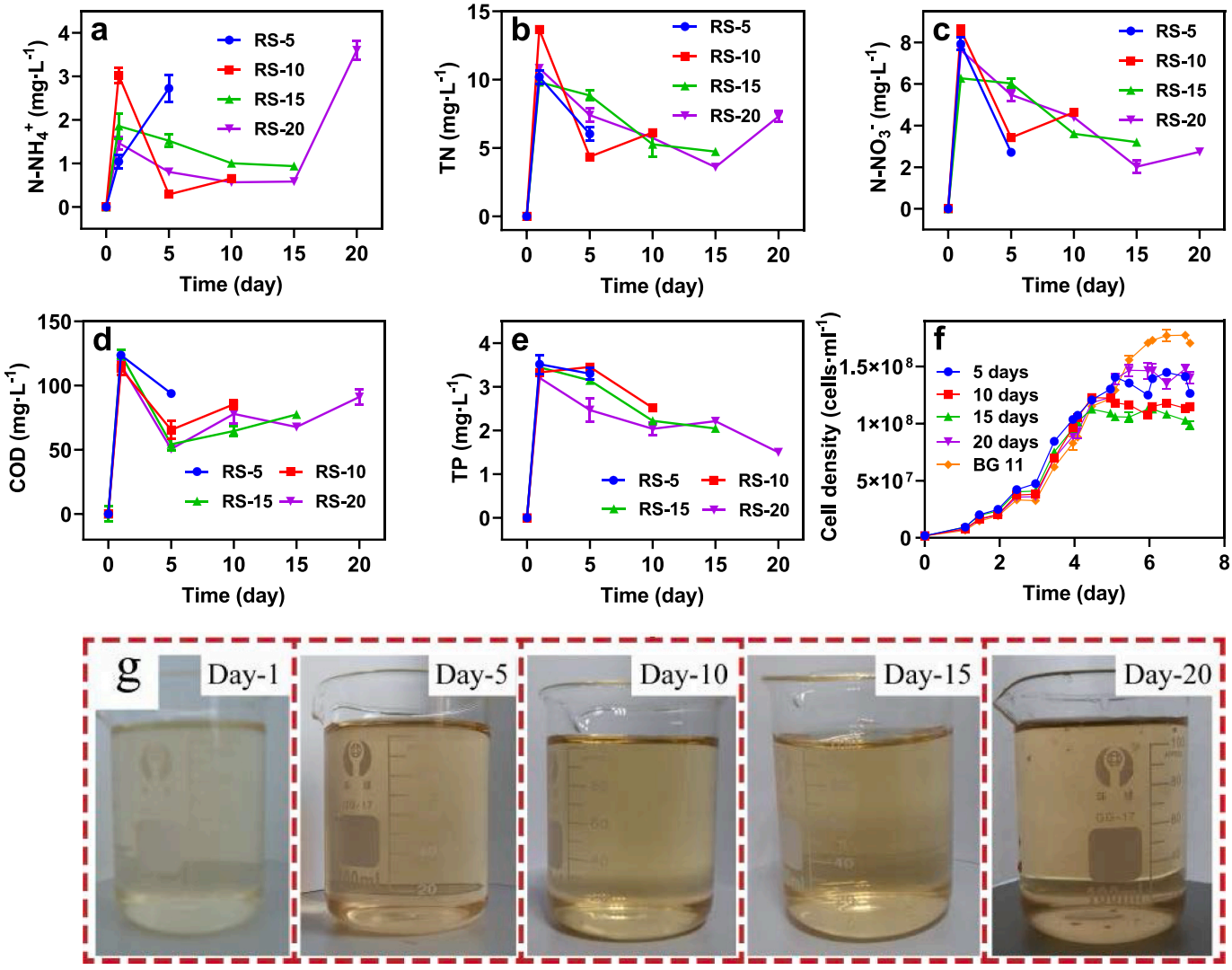

Fig. 4. Concentrations of N–NH_4^+ (a), TN (b), N–NO_3^- (c), COD (d), and TP (e) in the leachate from different treatments during the bioleaching process of rice straw; growth curves of the suspended *Chlorella sorokiniana* with BG 11 medium and leachate from different treatments (5 days, 10 days, 15 days, 20 days) as culture medium (f), the pictures of leachate collected at day 1, 5, 10, 15 and 20 from different treatments (g).

related to the organic metabolites produced by the indigenous microorganisms like acetic acid, propanoic acid, butanoic acid, monohydric alcohols, hydrolyzed sugars and glycerin during the biodegradation process of lignocellulosic materials (Lynd et al., 2002). Li et al. (2011) grew *Chlorella sorokiniana* on RS hydrolysate treated with trifluoroacetate and cellulase as a lignocellulose-based carbon source, and the biomass reached 2.83 g L^{-1} after 48 h of cultivation. However, after day 5, there was a significant variation in microalgal cell density among different culture media ($P < 0.05$), especially with BG 11 medium, which had a higher cell density than the leachates. Possibly reason lied in the fact that the concentration of TN and TP in the leachate was lower than BG 11 medium. Growth experiments with leachate as medium testified that indigenous microorganisms can decompose RS into soluble nutrients that can be used by microalgae to grow and reproduce during using unsterilized RS as microalgal biofilm carrier.

4. Conclusion

Unsterilized RS based microalgal biofilm exhibited enhanced biomass productivity and value-added products' production as the indigenous microorganisms mostly fugus effectively fixed *Chlorella sorokiniana* on it, and they could degrade RS into available nutrients for microalgae growth. Likewise, the RS experienced a pretreatment during the biofilm cultivation process by the indigenous microorganisms which carried by itself, and this further benefit its subsequent biological or thermochemical conversion to renewable energy material.

Credit author statement

Hongbin Yan: Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Writing - original draft. Qi Zhang: Conceptualization, Methodology, Formal analysis, Resources, Writing - review & editing. Yunpu Wang: Investigation, Formal analysis. Xian Cui: Conceptualization, Supervision, Writing - review & editing. Yuhuan Liu: Conceptualization, Writing - review & editing. Zhigang Yu: Investigation, Formal analysis. Shuming Xu: Formal analysis, Software. Roger Ruan: Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118075.

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