A biosurfactant-producing yeast *Rhodotorula* sp.CC01 utilizing landfill leachate as nitrogen source and its broad degradation spectra of petroleum hydrocarbons

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Abstract

In this study, a biosurfactant producing strain, *Rhodotorula* sp. CC01 was isolated using landfill leachate as nitrogen source, while olive oil was determined as the best sole carbon source for producing biosurfactants. The biosurfactant produced by *Rhodotorula* sp. CC01 was characterized as glycolipids with a critical micelle concentration of 70 mg/L, which showed stability over a wide range of pH (2−12), salinity (0−100%), and temperature (20−100°C). During the cultivation process, the surface tension decreased from 51.87 to 28.20 mN/m in 15 h, and the removal efficiency of NH_4^+-N reached 84.2% after 75 h cultivation with a maximum NH_4^+-N removal rate of 3.92 mg·L^{-1}·h^{-1}. In addition, *Rhodotorula* sp. CC01 has proven to be of great potential in remediating petroleum hydrocarbons, as revealed by chromogenic assays. The findings of this study prove a cost-effective strategy for the production of BS by yeast through the utilization of landfill leachate.

Introduction

Surfactants are amphiphilic compounds containing both hydrophilic and hydrophobic moieties (Ashitha et al., 2020; Markande et al., 2021; Sun et al., 2019). These moieties can reduce the surface and interfacial tensions and improve the solubility of hydrophobic compounds, thereby increasing the mobility and bioavailability of hydrophobic substrates (Ashitha et al., 2020; Derguine-Mecheri et al., 2021; Junhui Zhang, 2018). Surfactants can be synthesized by chemical routes, such as synthetic chemical surfactants, or produced by microorganisms, which are surface-active metabolites known as biosurfactant (BS) (Ahmed M. Eldin, 2019). Chemical surfactants tend to cause secondary pollution in soil and water, and pose potential hazards to human health and the environment (Derguine-Mecheri et al., 2021). Compared with chemical surfactants, BSs have more advantages, such as renewability, low toxicity, high biodegradability, and high environmental compatibility (Femina Carolin et al., 2021; Nogueira Felix et al., 2019; Rufino et al., 2011). In fact, surface tension could be reduced at lower concentrations of BS, making them more effective than chemical surfactants (Carolin et al., 2021). Overall, biosurfactant is a promising alternative for chemical surfactants (Femina Carolin et al., 2021; Nogueira Felix et al., 2019).

A large number of microorganisms have been reported to be capable of producing BS (Markande et al., 2021), such as *Sphingobacterium* (Burgos-Diaz et al., 2011), *Bacillus* (Sharma & Pandey, 2020), *Pseudomonas* (Sun et al., 2019), *Paracoccus* (Xu et al., 2020), *Acinetobacter* (Zhou et al., 2020), and *Wickerhamomyces anomalus* (Teixeira Souza et al., 2018). Due to the high production cost, complicated purification process, and low yield, the mass production of BS is limited (Carolin et al., 2021). A large proportion of BS production cost comes from raw materials, and it is a good strategy to produce BS using waste resources instead of traditional raw materials, which can significantly reduce production costs (Dobler et al., 2020). Microorganisms require suitable carbon and nitrogen sources which are essential for the production and synthesis of BS. (Aparna et al., 2012; Datta et al., 2018; He et al., 2020; Rosas-Galvan et al., 2018). To achieve this, various carbon sources such as molasses, whey, glycerol, orange peelings,

coconut oil cake, wastewater from olive oil mills, and crude glycerin were explored. (Aparna et al., 2012; Derguine-Mecheri et al., 2021; Dobler et al., 2020). Meanwhile, desirable nitrogen sources such as residual brewery yeast and urea were also explored for BS production. (Fonseca et al., 2007). However, few studies have worked on reducing the production cost of BS by replacing traditional nitrogen sources with NH_4^+-N-rich wastewaters, such as landfill leachate.

Landfill leachate is a type of wastewater comprised of rainwater, snow water, and moisture seepage from garbage landfills (Wu et al., 2016). Because of the complex composition, high organic matter and NH_4^+-N concentrations, landfill leachate poses great threats to ecosystems, including surrounding soils, rivers, groundwater, and the ocean environment (Koc-Jurczyk & Jurczyk, 2017; Ren et al., 2017; Wu et al., 2020; Yuan et al., 2019). On the other hand, compared with chemical and physical methods, biological treatment is a better choice for landfill leachate treatments due to the cost-effectiveness, environmental friendliness and feasibility (Yu et al., 2014; Zhang et al., 2020). However, high concentrations of NH_4^+-N in landfill leachate leads to poor biodegradability and low C/N ratio and presents a great challenge to biological methods (Yu et al., 2014). Owing to the aforementioned problems, the removal of NH_4^+-N from landfill leachate is necessary. Currently, NH_4^+-N pretreatment strategies mainly include nitrogen blowing, coagulation, electrocoagulation, oxidation, photocatalysis, and so on (Pirsaheb et al., 2015; Wang et al., 2003; Yu et al., 2014). However, most of these treatments are cost-consuming and can potentially cause secondary pollution (Yu et al., 2014). Using landfill leachate as a nitrogen source to produce BS can be a promising alternative to alleviate the nitrogen pollution.

Besides the landfill leachate problem, many petroleum hydrocarbons leakage occurs every year (Priya et al., 2015). Improper treatments threaten the environment through soil, groundwater, and ocean pollution (Sood & Lal, 2009). Biodegradation is a potentially powerful remediation approach to decontaminate oil pollutants since petroleum hydrocarbons occur naturally in the environment, and numerous microorganisms possess ability to utilize them as carbon sources and energy for growth (Yang et al., 2020). To date, studies on the application of biosurfactant-producing microorganisms to oil pollution have been extensively reported (Wei et al., 2020; Yang et al., 2020). However, few studies have reported the application of BS produced by *Rhodotorula* in these processes.

Hence, the main objectives of this study were to: 1) isolate BS-producing yeast using landfill leachate as nitrogen source and characterize the properties of the corresponding biosurfactants produced; 2) investigate the effect of the strain on nitrogen removal in landfill leachate; and 3) explore the effect of the strain on petroleum hydrocarbon utilization. This study is the first attempt to produce biosurfactants using landfill leachate as the nitrogen source. This could provide insightful information for the development of nitrogen removal strategy from landfill leachate.

Materials And Methods

Isolation of BS-producing strain

The landfill leachate used for the isolation of BS-producing strain was collected from Tuan Jishan Island of Zhoushan, China (122 °6 '4 "N, 29 °58 '15 "E). About 1 mL of landfill leachate was added into 200 mL of mineral salt medium (MSM) supplemented with 1% olive oil. The composition of MSM (pH 6.5–7.0) was the same as previously reported (Zhou et al., 2015). The culture was incubated at 30°C with shaking (180 rpm) for 3 days. Then, the culture suspension was scribed in blood agar plate (Ohadi et al., 2017) and blue agar plate (Sun et al., 2019), and incubated overnight. The isolates, which formed halos around their colonies, were incubated in the MSM with 1% olive oil as the sole carbon source. Ultimately, the culture supernatants were collected to evaluate the oil spreading performance and surface tension (ST). Briefly, 200 mL distilled water was poured in a petri plate (20 cm), followed by the addition of 400 μL crude oil to the surface of the water (Huang et al., 2020a). Then, 10 μL of cell-free supernatant was added to the center of the oil film, the MSM without inoculum was used as a control. The diameter of the clear zone was measured immediately. For ST measurement, 10 ml of the supernatant was taken in a petri dish and analyzed using a tension-meter (BZY 201, Shanghai Fangrui Instrument Co.Ltd, China) at room temperature.

Molecular identification of the isolated yeast

The strain with the best performance was selected for molecular identification. The genomic DNA was obtained using a EasyPure® Genomic DNA Kit (purchased from TransGen Biotech). The extracted DNA was further subjected to polymerase chain reaction (PCR) with ITS rRNA universal primers of ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The DNA sequence was compared with that of other microorganisms by BLAST. A phylogenetic tree was constructed using MEGA 7.0 software via the neighbour-joining method (Huang et al., 2020a).

Optimization of BS-producing conditions

Eight carbon sources were individually added to MSM (1% v/v) as the sole carbon source, including yeast extract, sodium acetate, glucose, *n*-hexadecane, olive oil, glycerol, diesel oil and paraffin. After 3 days of incubation (30°C, 180 rpm), the cell-free supernatant was collected by centrifugation at 8000 rpm for 5 min, then further characterized through ST and growth (OD_{600} nm) evaluations.

Growth and nitrogen removal performance of Rhodotorula sp.CC01

The *Rhodotorula* sp.CC01 activated overnight in yeast extract peptone dextrose medium was inoculated into the fermentation medium (1%, v/v), and cultured at 30°C and 180 rpm. The compositions of fermentation medium (pH 6.5–7.0) were as follows (g/L): NaCl, 15; KH_2PO_4, 0.5, K_2HPO_4, 1; $MgSO_4$, 0.5; KCl, 0.01; olive oil, 10; 10 mL of landfill leachate and 1 mL of trace elements (Zhou et al., 2015). Growth (OD_{600} nm), ST, NH_4^+-N, NO_2^--N and NO_3^--N concentrations of the culture were measured at intervals to evaluate the growth potential and nitrogen removal performance. The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were determined via a fully automatic chemical analyzer (Cleverchem380G, DeChem-Tech. GmbH, Germany) after filtration through 0.22 μm pore size nylon filters (Bkmam, Changde, China).

Extraction and performance evaluation of the BS

The BS extraction was performed according to the methods previously described by Zhou et al. (Zhou et al., 2020). Briefly, the culture broth was centrifuged at 8500 rpm for 5 min to obtain cell-free supernatant. Thereafter, 200 mL ethyl acetate was added to the precipitate. Subsequently, the organic phase was separated and concentrated by evaporation in the rotary vacuum evaporator to obtain the crude BS. For drop-collapse test, 1 ml of the culture supernatant was dyed with 100 µl of 0.003% methylene blue solution, and 50 µl of the mixture was dropped onto parafilm. After 1 min, the droplet collapse was observed. MSM medium was used as a control. For CMC determination, the BS was dissolved in distilled water at concentrations ranging from 20 mg/L to 200 mg/L. ST was measured at each concentration until a constant value was reached. CMC was determined by plotting ST as a function of the BS concentration. For stability analysis of BS, the BS solution of 100 mg/L was treated at different temperatures (i.e., 20, 40, 60, 80, and 100°C), salinity (i.e., 0, 20, 40, 60, 80, and 100 g/L), pH (i.e., 2, 4, 6, 8, 10, and 12) and the ST measurements were repeated three times.

Structure analysis of the BS

The structural property of the BS was investigated through thin layer chromatography (TLC), fourier transform infrared spectrum (FTIR), and gas chromatography−mass spectrometry (GC−MS) analysis. For TLC analysis, BS was dissolved in petroleum ether, and then absorbed by capillary tube. About 2 µL of solution was spotted on the silica gel plate (Merck, Darmstadt, Germany). The mobile phase of the petroleum ether/ethyl acetate (2:1, v/v) was used to separate the compounds. Iodine, 10% phosphomolybdic acid solution, 0.25% ninhydrin solution, and phenol-ammonium sulfate, was used to detect lipids, phospholipid, peptide, and carbohydrate, respectively. FTIR (IS10, Thermo-Nicolet, America) was used to analyze the surfactant in the spectral region of 4000 cm^{-1}−400 cm^{-1} to detect the characteristic functional groups in the sample. The composition and structure of fatty acids in BS were analyzed by GCMS according to the methods as previously reported (Zhou et al., 2020).

2,6-dichlorophenolindophenol (2,6-DCPIP) test

The *Rhodotorula* sp.CC01 cultured overnight was centrifuged and resuspended with 0.9% saline solution to remove the medium components. Substrates used in the experiment are as follows: cyclooctane, *n*-decane, *n*-hexadecane, octadecane, phenanthrene, light crude oil, heavy crude oil, and liquid paraffin. Prepare aqueous solution by dissolving Octadecane and phenanthrene in petroleum ether respectively. The reaction mixture contained approximately 750 µL MSM medium, 200 µL 2,6-DCPIP solution (37.5 mg/L), 50 µL $FeCl_3·6H_2O$ solution (150 mg/L), 200 µL cell suspension, and 10 µL sterilized substrate. The reaction was performed in an incubator (30°C, 180 rpm) for 72 h to observe the color change.

Statistical analyses and calculations

The NH_4^+-N, NO_2^--N and inorganic TN removal ratios were calculated as follows: $(c_0 − c_1)/c_0 × 100\%$, where c_0 is the initial NH_4^+-N/ NO_2^--N/ inorganic TN concentration and c_1 is the final concentration

(Wang et al., 2021; Zeng et al., 2020). The sum of NH_4^+-N and NO_3^+-N, NO_2^--N was defined as inorganic TN (Lu et al., 2019). The data generated in this study were analysed using Microsoft Excel and SPSS 13.0. Graphs were prepared using Origin 9.1.

Results

Isolation and identification of BS-producing strains

As shown in Table 1, four strains with BS-producing ability were isolated. Among them, the BS produced by the yeast strain CC01 showed the best performance. The CC01 supernatant exhibited the largest oil displacement diameter and the lowest ST (Table 1). Generally, the larger the oil spreading diameters, the better the activity of BS (Zhou et al., 2020). The efficiency of the yeast strain CC01 for the production of BS was confirmed by the reduction in ST (from 71.99 to 34.77 mN·m^{-1}), a flattened drop, as well as spread oil positive with a displacement zone diameter of 19.9 ±0.1 cm (Table 1 & Fig. 1b). Morphology of the yeast strain CC01 in LB agar plate was shown in Fig. 1b. The strain was identified as *Rhodotorula* sp, whose phylogenetic tree was constructed and presented in Fig. 1c. The ITS rRNA gene sequence of CC01 has been submitted to GenBank with an accession number MZ950605.

Table 1
Characterization of the isolated strains with BS-producing ability

Isolates	Oil displacement diameters (cm)	ST (mN/m)
CC06	1.00±0.50	51.85±0.44
CC04	2.50±1.00	50.34±0.20
CC01	19.90±0.10	34.77±0.63
CB02	1.95±0.65	54.30±0.30

Effect of carbon sources on BS production

Biosurfactant production is affected by the culture medium, in which carbon, nitrogen and trace element sources are essential to promote production (Nazareth et al., 2021). Among the eight carbon substrates examined, olive oil (1%, w/v) exhibited the best performance, with the corresponding ST of the culture medium dropped to 27.66 mN/m (Fig. 2a). Meanwhile, the highest growth of *Rhodotorula* sp.CC01 was also observed with olive oil as carbon source, suggesting that the reduction in ST positively correlated with the cell growth (Fig. 2a).

Growth kinetics and BS production of *Rhodotorula* sp.CC01

After establishing the optimum carbon source for cultivation, the growth kinetics and BS production (demonstrated by surface tension) were analyzed. As evident from the results displayed, the adaptive phase of *Rhodotorula* sp.CC01 occurred in the first 39 h, followed by the exponential phase prolonged

until 123 h (Fig. 2b). Meanwhile, ST of the culture reduced from 51.87±1.74 to 28.20 ± 0.51 mN/m after 15 h of cultivation during the early exponential and remained stable throughout the incubation period of 243 h.

Nitrogen removal performance of *Rhodotorula* sp.CC01

The landfill leachate used in this study is rich in nitrogen sources with ammonium concentration as high as 3000 mg/L. Therefore, it was supplemented to the medium as nitrogen sources at the ratio of 1% (V/V) for the production of BS by *Rhodotorula* sp.CC01. The ability of *Rhodotorula* sp.CC01 to assimilate different nitrogen sources in the medium was further investigated. It could be observed that with the growth of the strain CC01, a decrease in NH_4^+-N occurred immediately and 87.5% of NH_4^+-N was removed in 75 h and the maximum NH_4^+-N removal rate was 3.92 mg·L^{-1}·h^{-1} (Fig. 3a). Similarly, while NH_4^+-N was rapidly removed, the removal efficiency of NO_2^--N reached 71.7% (Fig. 3b). In addition, it is worth noting that significant fluctuations in NO_3^--N were observed during the process (Fig. 3c), which is probably attributed to heterotrophic nitrification. Finally, the removal efficiency of inorganic TN reached 80.6% after 75 h cultivation, and strain CC01 proliferated without a lag phase (Fig. 3d), suggesting that nitrogen might be mainly assimilated to organic matter (Civiero et al., 2018).

Properties of produced BS

In this study, the CMC of the BS produced by *Rhodotorula* sp.CC01 was 70 mg/L and the ST was 33.39 mN/m (Fig. 4a). Meanwhile, the BS exhibited stable surface activity under different pH conditions (Fig. 4b). Similarly, the ST of BS had no significant change with salinity ranging from 0 to 100 mg/L (Fig. 4c). In addition, the BS also showed excellent stability over a wide temperature range (Fig. 4d).

TLC analysis showed that the BS produced by *Rhodotorula* sp.CC01 is completely separated using different solvent systems and shows three points (R_{f1}= 0.18, R_{f2}=0.70, R_{f3}=0.87) (Fig. 6a). Further chromogenic reaction analysis revealed that the BS contained lipids, peptides, and sugar compounds (Fig. 5a-c), demonstrating that it would be categorized as glycolipoprotein.

FTIR analysis revealed that BS and standard rhamnolipid were fairly similar (Fig. 5d). The primary difference observed in BS and standard rhamnolipid was the bands of 3371 cm^{-1} (O-H stretch) (Sen et al., 2017). The bands observed at wavenumber 2923cm^{-1} and 2853cm^{-1} were attributed to the characteristic C-H stretching and vibration, indicating the existence of methyl and methylene groups, respectively (Ashitha et al., 2020; Liu et al., 2014) (Fig. 5d). The peak at 1745cm^{-1} corresponded to C=O stretching of lactones and the absorption bands at 1464cm^{-1} were caused by C-H stretching and vibration of fatty acid group (Ashitha et al., 2020; Burgos-Diaz et al., 2011; Xiao et al., 2013). The absorption bands observed at 1161cm^{-1} demonstrated the existence of C-O and C-O-C bonds of the carboxylic acids, and the bands at 1377 cm^{-1} revealed the presence of C−N bonds (Derguine-Mecheri et al., 2021; Sen et al., 2017). Above all, FTIR result revealed the presence of aliphatic chains and peptide moieties.

The BS of *Rhodotorula* sp.CC01 after methyl esterification and hydrolysis was analyzed by GC−MS in order to identify the fatty acid types (Ashitha et al., 2020). Four peaks were observed through GC-MS analysis, which were close to methyl hexadecanoate (C16:0), methyl octadecanoate (C18:0), methyl octadecanoate (C18:1), and methyl octadecanoate (C18:2) (Fig. 5e).

Petroleum hydrocarbon utilization range by *Rhodotorula* sp.CC01

It could be observed that the color of the reaction group containing cyclooctane as the sole carbon source became colorless. This is also the case for other sole carbon sources such as *n*-decane, *n*-hexadecane, octacosane, phenanthrene, light crude oil, heavy crude oil, and paraffin, respectively (Fig. 6). Meanwhile, colors of control groups remained blue (Fig. 6). Thus, it could be considered that *Rhodotorula* sp.CC01 had a wide utilization range of petroleum hydrocarbon including short chain alkanes, medium chain alkanes, long chain alkanes, mixed crude oil, PAHs, and liquid paraffin.

Discussion

In this work, innovative approaches have been developed and applied as strategies to valorize landfill leachate as a nitrogen source for low-cost biosurfactant production by the yeast *Rhodotorula* sp.CC01. The carbon source of the culture medium was optimized and the olive oil showed better performance. A previous study also reported the promotion of BSs production by microorganisms cultivated in medium containing olive oil (Huang et al., 2020b). Meanwhile, Teixeira et al.(2018) evaluated BS production by *Wickerhamomyces anomalus* CCMA 0358 yeast and clarified olive oil as an essential carbon source. Derguine et al. (2021) also investigated BS production by *Rhodotorula* sp.YBR using olive oil mill wastewater as substrate for low-cost production, and the produced BSs showed good surface activity with a yield of 10.08 ± 0.38 g L^{-1}.

Incubation experiment showed that ST reduction was observed after 15 h when growth occurred at the adaptive phase, indicating that the strain can efficiently utilize olive oil and landfill leachate to produce BS in a short time (Fig. 2b). The biomass increased with incubation time and reached a maximum at 147 h, whereas the ST remained steady till the end of the cultivation period. Similar growth-associated production of BS was also observed by Suparna et al. (2017). However, Santos et al. (2017) reported that the highest reduction in ST due to BS production by *Streptomyces* sp. DPUA 1559 was observed after 60 h during the stationary growth.

The results of nitrogen removal analysis showed that the removal efficiency of NH_4^+-N was 87.5% and the maximum NH_4^+-N removal rate was 3.92 mg·L^{-1}·h^{-1}, which was significantly higher than that of *Acinetobacter tandoii* MZ-5 (2.28 mg·L^{-1}·h^{-1}) (Ouyang et al., 2020), *Pseudomonas tolaasii* Y-11(2.04 mg·L^{-1}·h^{-1}) (He et al., 2016), and *Bacillus methylotrophicus* L7 (2.15 mg·L^{-1}·h^{-1}) (Zhang et al., 2012). Similarly, while NH_4^+-N was rapidly removed, the accumulation of NO_3^--N was observed for the red yeast *Sporidiobolus pararoseus* Y1 while NO_2^--N accumulation was not evident (Zeng et al., 2020).

Generally, reducing ST (below 35 mN/m) is one of the critical indicators for selecting BS-producing microorganisms (Chandankere et al., 2014). Thus, the CMC of the BS in this study was compared with that produced by other yeasts and bacteria reported in literature (Table 2). The results showed that the BS produced by *Rhodotorula* sp.CC01 had much lower CMC than others, indicating higher aggregation ability and better surface activity (Aparna et al., 2012; Derguine-Mecheri et al., 2021; Huang et al., 2020b; Luna et al., 2013; Sen et al., 2017).

Table 2
Comparision of BS produced by different strains

Strain	CMC(mg/L)	ST(mN/m)	Types of BS	Reference
Pseudomonas sp. 2B	100	29.73	Glycolipids	(Aparna et al., 2012)
*Serratia marcescens*ZCF25	220	29.50	lipopeptide	(Huang et al., 2020b)
Rhodotorula sp. YBR	180	30.16	Glycolipids	(Derguine-Mecheri et al., 2021)
Rhodotorula babjevae YS3	130	35	Sophorae lipid	(Sen et al., 2017)
Candida sphaerica UCP0995	250	25	Glycolipids	(Luna et al., 2013)
Rhodotorula sp.*CC01*	70	33.39	Glycolipids	This study

The BS produced by the yeast *Rhodotorula* sp.CC01 was classified as glycolipoprotein by TLC. Similarly, Derguine et al. reported that the BS produced by *Rhodotorula* sp. YBR was characterized as glycolipoprotein, including carbohydrate (R_f = 0.62), protein (R_f = 0.48) and lipid (R_f = 0.76) (Derguine-Mecheri et al., 2021). The type of the BS produced by *Rhodotorula babjevae* YS3 was determined as sophorolipid (Sen et al., 2017). Further FTIR analysis showed that the BS produced by the strain CC01 exhibited similar functional groups with standard rhamnolipid, which are in agreement with those obtained with TLC. Moreover, the BS of *Rhodotorula* sp.CC01 was mainly hexadecanoic acid and octadecenoic acid by GC-MS analysis.

Conclusion

This study reported the isolation and identification of an indigenous BS-producing yeast strain from landfill leachate. The strain *Rhodotorula* sp.CC01 produced BS using landfill leachate as its nitrogen source, and the removal efficiency of NH_4^+-N was 84.2% after 75 h cultivation. The strain opens new future prospects for BS produced at lower costs as an alternative to chemical surfactants and nitrogen

removal from landfill leachate. Meanwhile, *Rhodotorula* sp.CC01 exhibits a broad utilization spectrum of petroleum hydrocarbons, suggesting a promising prospect in the remediation of petroleum hydrocarbon pollutants.

Declarations

CRediT authorship contribution statement

Xiaoyun Lin: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft; **Hanghai Zhou**: Investigation, Formal analysis, Data curation; **Feng Zeng**: Investigation; **Lijia Jiang**: Investigation; **Edidiong Okokon Atakpa**: Manuscript revision; **Chunfang Zhang**: Validation, Writing - review & editing, Project administration; **Qinglin Xie**: Review & editing.

Declarations of 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.

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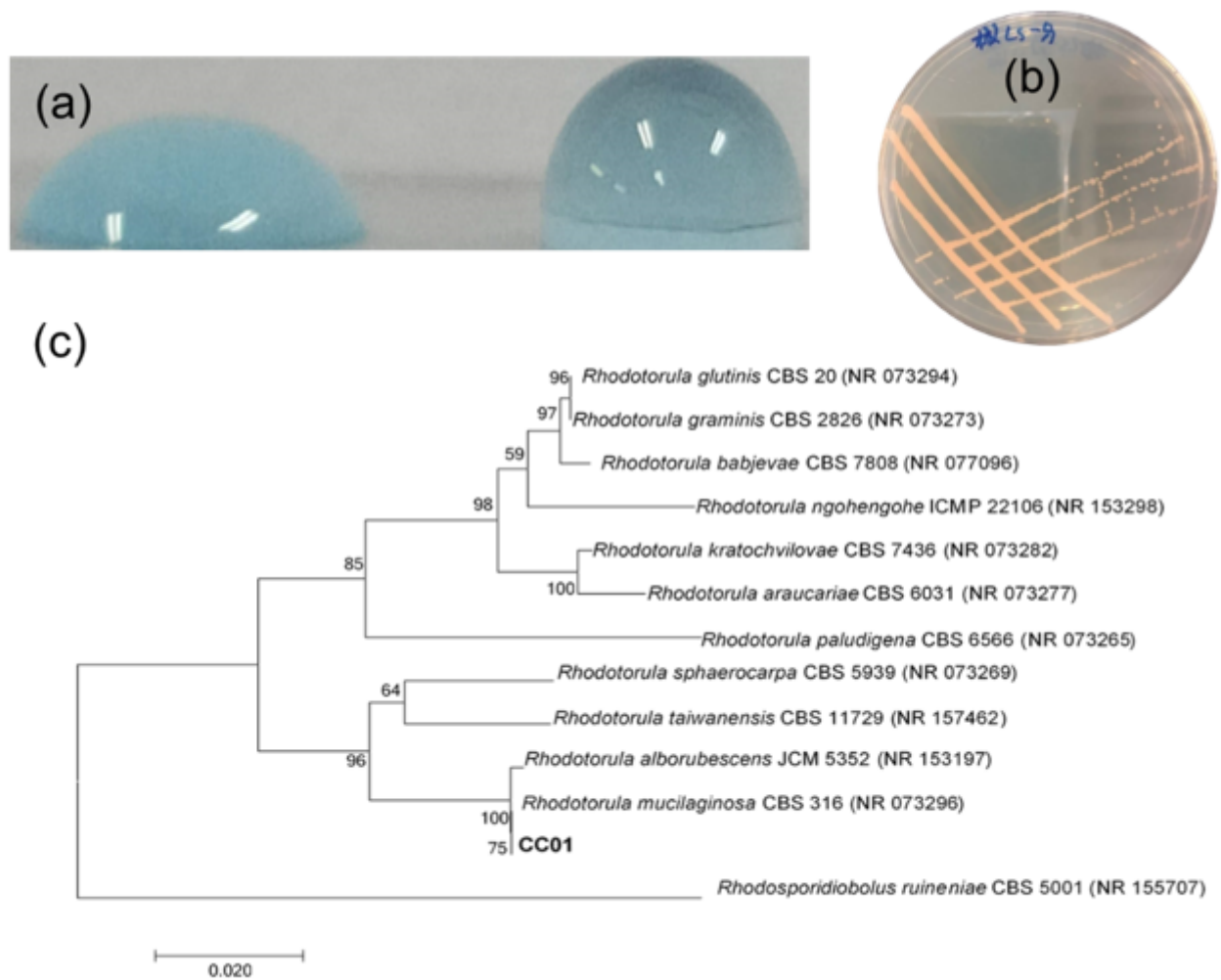
Figures

Figure 1

Drop-collapse test (a), morphological characteristics under plate (b), phylogenetic relationship (c) of strain CC01 isolated from landfill leachate. In Fig. a, right image represents control while left image represents cell-free supernatant. In Fig. c, the tree is constructed using ITS rDNA gene sequence using neighbour-joining method.
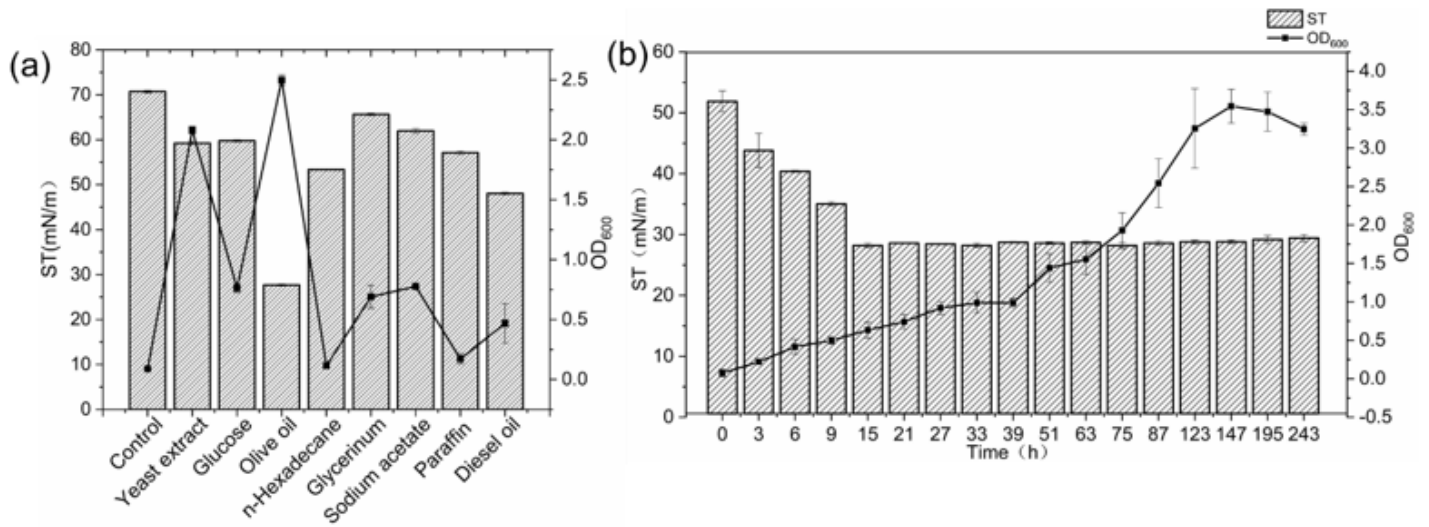
Figure 2

(a) Effects of eight carbon sources on ST and OD600 of strain CC01 after 72 hours of incubation. (b) Kinetics of growth and ST of strain CC01 during 243 hours of incubation. The error bars represent the standard deviations.
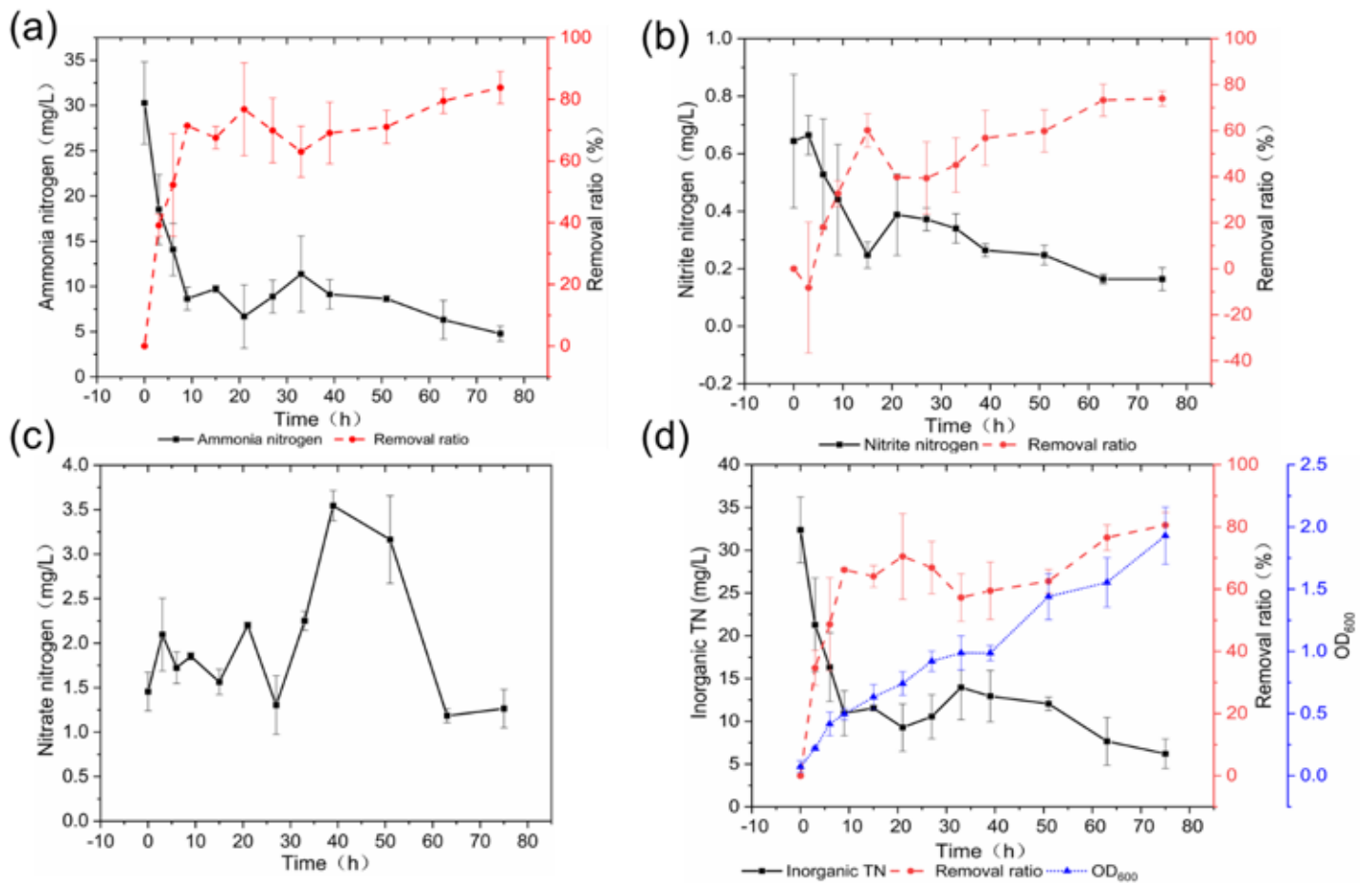
Figure 3

The removal performance of NH4+-N (a), NO2–N (b), NO3–N (c) and inorganic TN (d) in landfill leachate by strain CC01 during 75 h cultivation. The error bars represent the standard deviations.
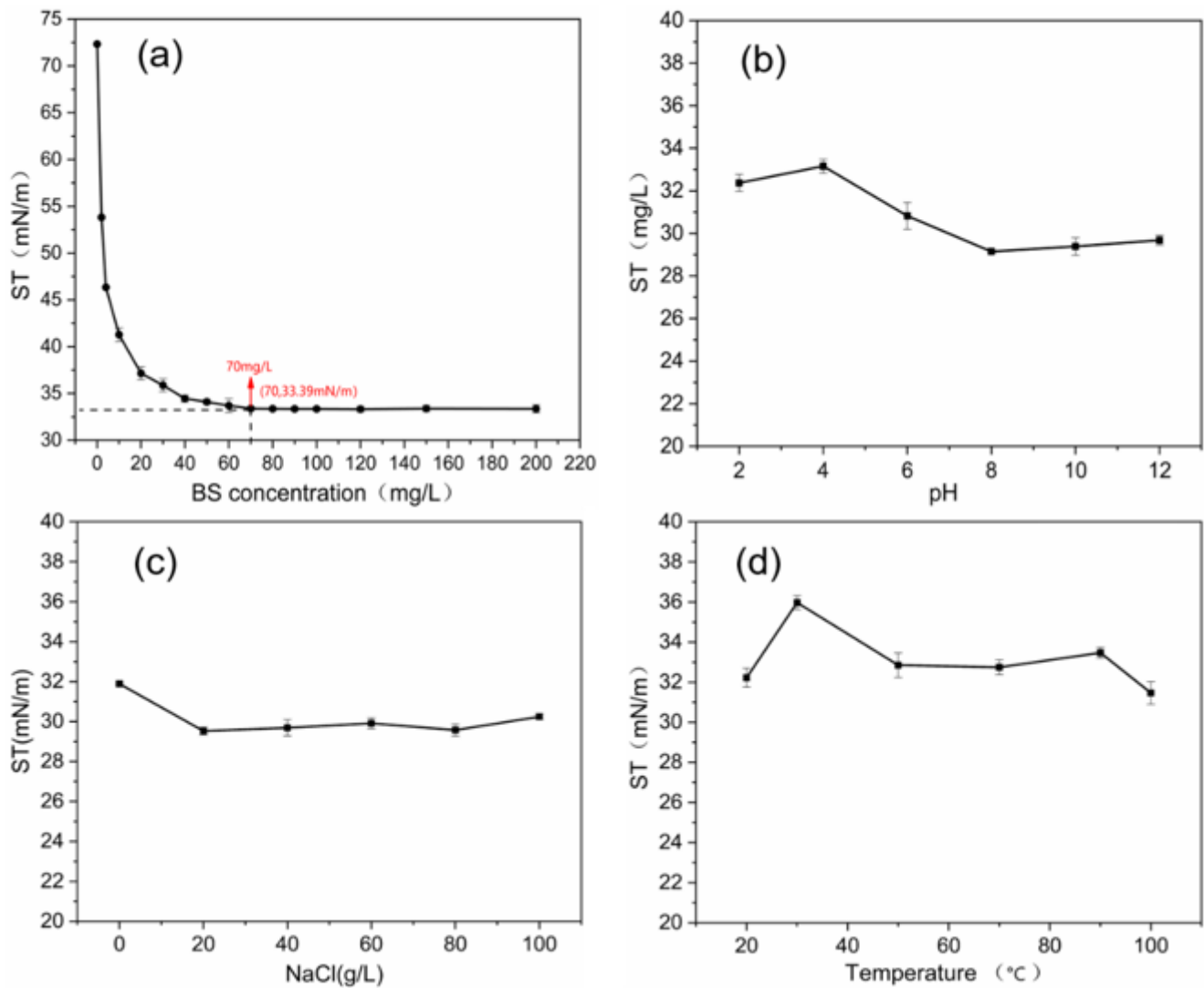

Figure 4

The CMC determination of the BS produced by strain CC01 (a); The BS stability against different pH (b), salinity (c), and temperature (d). The error bars represent the standard deviations.
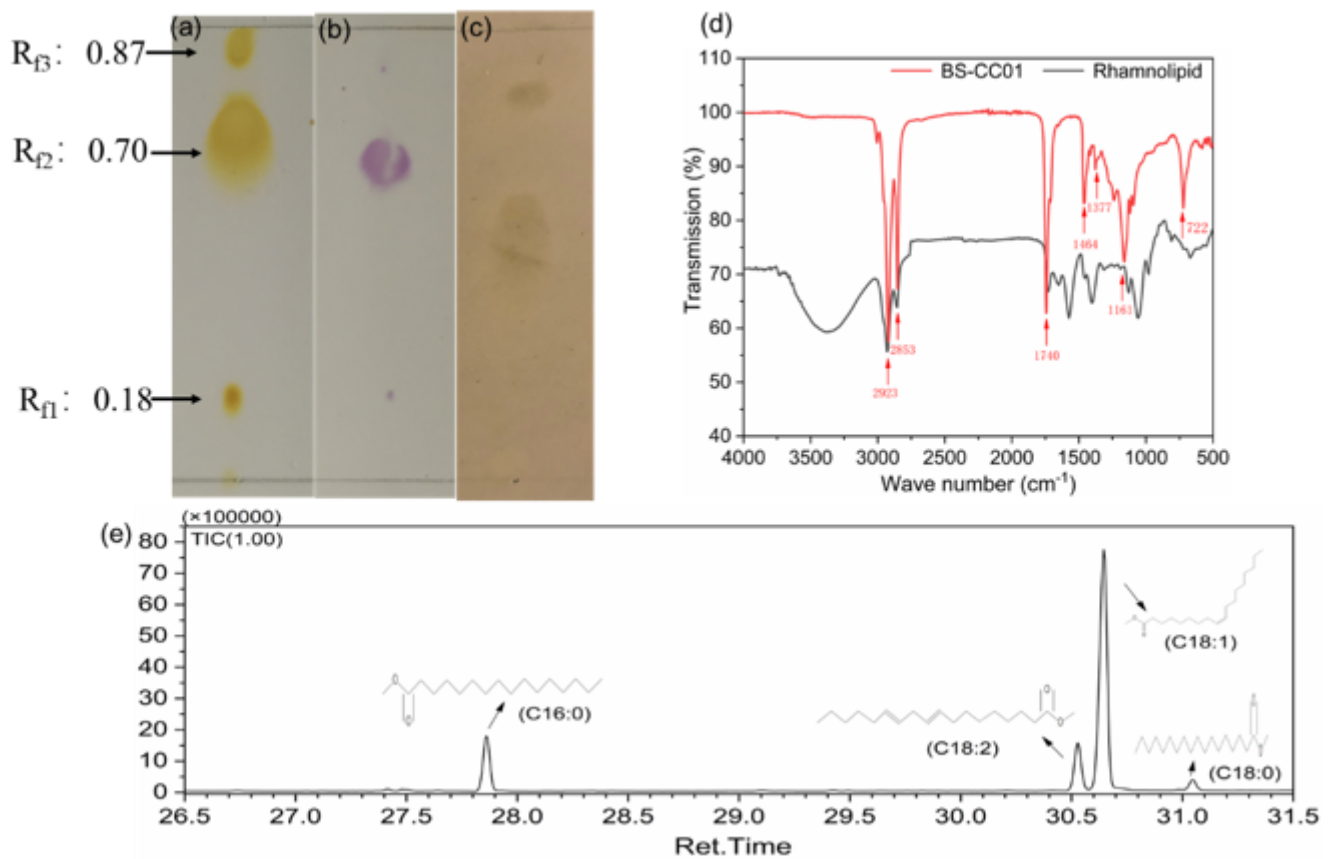
Figure 5

TLC (a-c), FTIR spectra (d), and GC−MS (e) analysis of the BS produced by strain CC01. In Fig. a-c, a image represented iodine vapours of color detection. The citric yellow is positive. B image represented the color detection spraying ninhydrin solution to detect peptide content. C image represented the color detection spraying sulfuric acid phenol solution to detect sugar compounds.
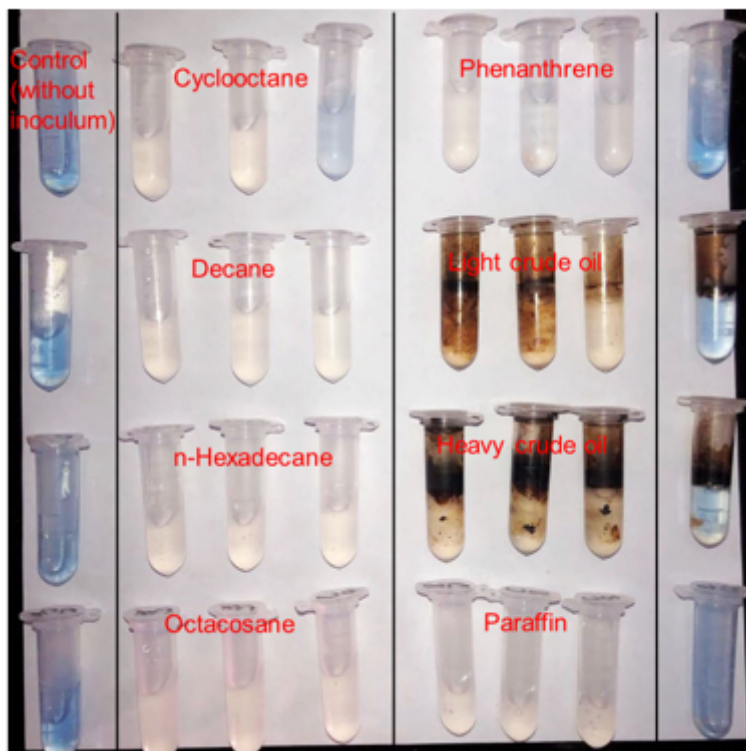
Figure 6

2,6-DCPIP experimental results of strain CC01

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