1 **Biological Conversion of the Aqueous Wastes from**
2 **Hydrothermal Liquefaction of Algae and Pine Wood**
3 **by *Rhodococci***

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11

Abstract

In this study, *R. opacus* PD630, *R. jostii* RHA1, *R. jostii* RHA1 VanA⁻, and their co-culture were employed to convert hydrothermal liquefaction aqueous waste (HTLAW) into lipids. After 11 days, the COD reduction of algal-HTLAW reached 93.4% and 92.7% by *R. jostii* RHA1 and its mutant VanA⁻, respectively. Woody-HTLAW promoted lipid accumulation of 0.43 g lipid/g cell dry weight in *R. opacus* PD630 cells. Additionally, the total number of chemicals in HTLAW decreased by over 1/3 after 7 days of coculture, and 0.10 g/L and 0.46 g/L lipids were incrementally accumulated in the cellular mass during the fermentation of wood- and algal-HTLAW, respectively. The GC-MS data supported that different metabolism pathways were followed when these *Rhodococci* strains degraded algae- and woody-HTLAW. These results indicated promising potential of bioconversion of under-utilized carbon and toxic compounds in HTLAW into useful products by selected *Rhodococci*.

Key words: Hydrothermal liquefaction aqueous waste; Lipid; Bioconversion; Coculture; Oleaginous *Rhodococci*

1. Introduction

Intensified efforts have been applied in recent years to develop biological and thermochemical processes for biomass conversion to liquid fuels in order to reduce green-house gases, lower the nation's dependence on imported petroleum crude, create domestic biorefinery industries and employment opportunities. Hydrothermal liquefaction (HTL) is a thermochemical conversion process is receiving considerable interest due to its inherent simplicity, ability to handle a wide range of biomass feedstock (i.e., lignocellulosics, agricultural residues, wet wastes, and algae), and high carbon efficiency (Elliott et al., 2015). In HTL processes, aqueous biomass slurries are heated (200–400 °C) under pressure (10–20 Mpa) and held at target temperature for sufficient time to break down the solid biopolymeric structure into a mixture of products, including biocrude, gas rich in CO₂, solid residue (primarily ash), and an aqueous byproduct with high concentrations of both organics and nutrients (Elliott et al., 2013 & 2015). HTL can release nitrogen and oxygen from biomass into the aqueous and gaseous fractions. Carbon yields to biocrude often exceed 50% while carbon yields to the aqueous phase range from about 15% to 45% depending on the biomass source. The overall carbon efficiency of the HTL process can be significantly improved by converting the hydrothermal liquefaction aqueous waste (HTLAW) into useful products. Current options for utilization of HTLAW include gasification, anaerobic digestion, and nutrient recycle (for algal applications). However, the compositions of HTLAW present challenges to these options. The HTLAW streams contain high concentration of chemical oxygen demand (COD) and/or high concentration of ammonia (1.9-7.1 g/L) in addition to other characteristics that make HTLAW unsuitable for direct discharge to the environment (Pham et al., 2013). Therefore, it is important to develop novel approaches that effectively degrade HTLAW.

One widely applied approach of wastewater treatment is microbial degradation (Hollinshead et al., 2016; Li et al., 2013; Zeng et al., 2013). It is well-known that *Rhodococci* can catabolize short- and long-chain alkanes, aromatic (nitro-, hydroxyl-, halogenated substituted), heterocyclic and polycyclic aromatic compounds (Holder et al., 2013; Jena et al., 2011; He et al., 2012 & 2013; Labana et al., 2015; Larkin et al., 2005; Nadaf et al. 2014; Lenke, 1996; Muller et al., 2014). *Rhodococcus* UKMP-5M degraded 900 mg/L phenol in wastewater (Suhaila et al. 2013). The maximum phenol oxidation achieved about 34%, promoting an increase in 28% of *R. erythropolis* growth, 10% of respiration activity with shorter lag- and exponential-phase interval (Křiklavová et al. 2014). *R. erythropolis* UPV-1 grown in synthetic wastewater significantly decreased 90% of COD, 98.7% of phenol and 99.7% of formaldehyde (Hidalgo et al. 2002). *Rhodococcus* sp. NCIM 2891 was also evaluated in the pulp and paper industrial effluent remediation, resulting in 53.2% decrease of COD, 81.0% reduction of the total dissolved solid along with a decrease in heavy metal concentrations (Nadaf et al., 2014). *Rhodococcus* sp. DK17 was isolated from soil and analyzed for the ability to grow on *o*-xylene as the sole carbon and energy source. Although DK17 couldn't grow on *m*- and *p*-xylene, it was capable of growing on benzene, phenol, toluene, ethylbenzene, isopropylbenzene, and other alkylbenzene isomers (Kim et al., 2002). *Rhodococcus* sp. EH831 was reported to degrade alcohols, chlorinated hydrocarbons, cyclic alkanes, ethers, ketones, monoaromatic and polyaromatic hydrocarbons, and petroleum hydrocarbons (Lee et al., 2010).

Recently, it was also reported that *Rhodococcus* strains are strong candidates to produce lipids using aromatic compounds (Kurosawa et al. 2013; Kosa and Ragauskas, 2013; Revellame, et al., 2013). Eukaryotic organisms are mainly known to store lipophilic compounds, which have higher calorific value than carbohydrates and proteins. Lipid accumulation was observed in

1 *Rhodococcus erythropolis*, *Rhodococcus fascians*, *Rhodococcus rhodochrous*, and *Rhodococcus*
2 *ruber* etc., which were capable of synthesizing triacylglycerol (TAG) as their carbon and energy
3 storage (Alvarez et al., 2000; Voss and Steinbüchel, 2001). Ethanol organosolv lignin and its
4 ultrasonicated product were found to be sufficient carbon sources for *R. opacus* DSM 1069 to
5 accumulate lipids up to 0.04 g lipid/g CDW (Kosa and Ragauskas, 2013). *R. opacus* PD630
6 could degrade vanillic acid via the β -ketoadipate pathway and biotransform vanillic acid to lipids
7 (Kosa and Ragauskas, 2012; Zhao et al., 2013). Laccase and *R. opacus* PD630 cells synergized
8 in lignin degradation, leading to a 17-fold increase of lipid production compared to that without
9 laccase addition (Zhao et al., 2016). In contrast, few reported effective reduction of COD in
10 HTLAW and bioconversion of HTLAW into lipids.

11 HTL is a promising and carbon efficient approach to produce petroleum-like biocrude from
12 biomass (Elliott et al., 2015). However, many polar organic compounds, including organic acids,
13 alcohols, ketones, and biocrude constituents, are produced during HTL (Elliott et al., 2013 &
14 2015). Biotransformation of HTL aqueous waste (HTLAW) to biofuel is highly challenging due
15 to potential inhibition and toxicity from the biocrude constituents along with its high COD. Thus,
16 the overall goal of this study is to develop a cost-effective biological process that converts the
17 organic carbons, including aromatics and other organic compounds in the HTLAW, into
18 microbial biomass enriched with lipids that can then be used for biofuel production. This strategy
19 will improve the carbon efficiency of thermochemical conversion of various biomass feedstocks.
20 In this study, three aerobic *Rhodococci* strains (e.g. *R. opacus* PD630, *R. jostii* RHA1 and its
21 mutant *R. jostii* RHA1 VanA⁻) with strong aromatics degradation and/or lipids biosynthesis
22 capacities were selected for investigation to reduce the HTLAW COD as well as to produce
23 lipids.

2. Materials and Methods

2.1. Chemicals and wastewater

Tryptic Soy Broth (BD, cat #: 257107) was obtained from St. Becton, Dickinson and Company (USA). All other chemicals were also from commercial sources and of analytical grade. Aqueous wastewater generated during HTL conversion of loblolly pine (WD38) and marine *Tetraselmis* algae (Tet06) were kindly provided by the Pacific Northwest National Laboratory (PNNL). The bench-scale continuous HTL system, which was used to generate WD38 HTLAW and Tet06 HTLAW, was previously described by Elliott et al. (2013). Table 1 shows the selected characteristics of the WD38 HTLAW and Tet06 HTLAW.

2.2. Microorganisms

R. opacus PD630 was kindly provided by Dr. Joshua Yuan (Texas A&M University) while *Rhodococcus jostii* RHA1 and *R. jostii* RHA1 VanA⁻ were generously supplied by Dr. Lindsay Eltis (University of British Columbia, Canada).

2.3. Fermentation of HTLAW

The strains were cultivated in Tryptic Soy Broth (BD, cat #: 257107) for 24 h at 30 °C and 180 rpm. The cells were harvested by centrifugation (8,000×g, 10 min) at 4 °C and washed twice with sterilized potassium phosphate buffer (KPB) (100 mM, pH 7.0). The cells re-suspended in the same KPB at OD₆₀₀=1.0 were used as inoculum while the supernatant was discarded after the final wash. To start single strain fermentation, 1% (v/v) inoculum was added to 100 ml HTLAW without added nutrition. To start fermentation of co-culture, 0.333 mL *R. opacus* PD630, 0.333

1 mL *R. jostii* RHA1, and 0.333 mL *R. jostii* RHA1 VanA⁻ were added to 100 ml HTLAW without added nutrition. The 100 ml nutrient medium was comprised of (per liter): 1.4 g (NH₄)₂SO₄, 1.0 g MgSO₄·7H₂O, 1.0 ml CaCl₂·2H₂O (15 g/L), 1.0 ml sterile trace element solution (Stock A). Stock A solution contains: NaMoO₄·H₂O 2.0 g/L and FeNa-EDTA 5.0 g/L. Fermentation experiments were carried out at 30°C and 180 rpm. 5 ml samples were taken during the cultivation for analysis.

2.4. Analysis of soluble compounds by GC-MS

After 10 ml fermentation broth was centrifuged at 9000 rpm for 30 min at 4 °C, the cells were discarded. The supernatant was filtered through a 0.2 µm[†] hydrophilic filter (Millipore Express® SHC) and then analyzed by GC/MS. The analysis utilized an Agilent 6850 series II GC system with an Agilent DB-Wax column (30 m by 0.32 mm, 0.5 µm thick film) with hydrogen as the carrier gas. A portion of the organic phase was injected with a 30:1 split ratio by an auto-sampler. The inlet was maintained at 250 °C. The oven was held at 80 °C for 5 min, heated to 220 °C at 20 °C/min, and then held at 220 °C for 5 min. Peak detection was performed by a flame ionization detector, which was maintained at 300 °C.

2.5. Extraction and analysis of cellular lipid

Cellular lipids were extracted following methods as described previously ([Kosa and Ragauskas, 2013](#)). To collect cells, 50 mL of fermentation broth was centrifuged at 9000 rpm for 10 min at 4 °C. The pellets were vortexed with 20 ml 100% methanol until the pellet was completely re-suspended, then were incubated at 65 °C for 30 min in a water bath. 1mL of 10 N KOH was added and vortexed for 1 min, and then incubated at 65 °C for 2 h. The samples were

1 cooled to room temperature followed by slow addition of 1 ml H₂SO₄, and then were incubated
2 again at 65 °C in a water bath for 2 h. To extract the lipids, 8 ml of hexane was added after the
3 samples cooled down to room temperature. Next, the samples were shaken 5 min and centrifuged
4 for 5 min at 3000 rpm. The hexane layer was collected and placed under vacuum for three days
5 to evaporate the solvent till constant weight, and then the amount of lipids was obtained by
6 weight measurement.

7 2.6. COD assay

8 **COD** was determined using Hach method 8000, Program 435 with a DR 2800™ Portable
9 Spectrophotometer. The range of the method is 20-1,500 mg O₂/L. The Hach method is USEPA
10 approved for waste water analysis, Standard Method 5220D.

12 2.7. Dry mass determination

13 After the cells were harvested from the fermentation broth by centrifuging at 9000 rpm for 30
14 min at 4 °C, the supernatant was discarded after centrifugation. Cells were harvested and washed
15 twice with 0.8 wt% NaCl solution. Cells were then dried at 60 °C in an oven for two days to
16 constant weight, and the dry weight was determined.

18 3. Results and Discussion

19 3.1. COD reduction and lipid accumulation with *Rhodococci* single strain culture

20 *Rhodococcus* strains have been effectively employed to remove COD of petrochemical
21 wastewater and para-nitrophenol wastewater (Rezouga et al., 2009; Sponza and Gök, 2010). In this
22 study, single strain cultures of *R. opacus* PD630, *R. jostii* RHA1, and *R. jostii* RHA1 VanA⁻ were
23 tested for the COD reduction in HTLAW samples. As shown in Fig. 1, these strains appreciably

decreased the COD of WD38 and Tet06 HTLAW. During the 48 h single strain culture, COD reduction of Tet06 HTLAW and WD38 HTLAW by *R. opacus* PD630 reached 43.0% and 50.3%, respectively. COD of Tet06 HTLAW and WD38 HTLAW were removed by *R. jostii* RHA1 VanA⁻ at 44.9% and 38.5%, respectively. COD reduction of HTLAW by *R. jostii* RHA1 reached 44.5% and 50.6% for Tet06 HTLAW and WD38 HTLAW, respectively. Clearly, *R. jostii* RHA1 had better capability for COD reduction of WD38 HTLAW than *R. opacus* PD630 and *R. jostii* RHA1 VanA⁻. For Tet06 HTLAW, *R. opacus* PD630, *R. jostii* RHA1, and *R. jostii* RHA1 VanA⁻ single culture showed similar COD reduction (43.0-44.9%) after 48 h. Furthermore, time courses for the bioconversion of Tet06 HTLAW by single *Rhodococcus* strain were monitored (Fig. 2). After 3 days, COD of Tet06 HTLAW were removed by *R. opacus* PD630, *R. jostii* RHA1, and *R. jostii* RHA1 VanA⁻ single culture at 56.6%, 77.6% and 77.8%, respectively. After 11 day of biodegradation, the COD value in Tet06 HTLAW by *R. opacus* PD630 was reduced by 85.1%. Notably, results showed that *R. jostii* RHA1 and *R. jostii* RHA1 VanA⁻ more effectively removed COD than *R. opacus* PD630. After the degradation with *R. jostii* RHA1 and its mutant VanA⁻ for 11 days, the COD values in Tet06 HTLAW were reduced by 93.4% and 92.4%, respectively.

Lipid accumulation has been observed in the **genus** *Rhodococcus*, such as *Rhodococcus erythropolis*, *Rhodococcus fascians*, *Rhodococcus ruber*, *Rhodococcus jostii* and *Rhodococcus opacus* (Alvarez et al., 2000; Kumar et al., 2015; Kosa and Ragauskas, 2013; Li et al., 2010; Revellame, et al., 2013; Voss and Steinbüchel, 2001). In this study, the lipid content and the bacterial cell dry weight (CDW) of the *Rhodococcus* cells were investigated (Fig. 3). Low lipid accumulations from 0.072 ~ 0.095 g lipid/g CDW were found when WD38 HTLAW was degraded by single culture of *Rhodococcus* sp. (Fig. 3a). In contrast, Tet06 HTLAW promoted as

high as 0.43 g lipid/g CDW of lipid accumulation in *R. opacus* PD630 cells after 11 days (Fig. 3b). In comparison, with raw dairy wastewater, *R. opacus* DSM 43205 accumulated 14.3% lipid (w/w) and reduced the wastewater COD by 30% (Kumar et al., 2015). It was found that the lipid accumulation in *R. opacus* DSM 1069 cells was 0.142 g lipid/g CDW using oxygen-pretreated Kraft lignin as carbon source (Wei et al., 2015). Results showed that Tet06 HTLAW as potential carbon source resulted in significant lipid accumulation in *R. opacus* PD630 cells.

3.2. GC/MS analysis of biodegradation products with single *Rhodococcus* strain culture

It was reported that *Rhodococci* could catabolize some alkanes, aromatic, heterocyclic and polycyclic aromatic compounds (He et al., 2013; Nadaf et al. 2014; Muller et al., 2014; Wei et al., 2015). In this study, GC/MS was applied to analyze biodegradation products of WD38 HTLAW with single *Rhodococcus* strain culture (Table 2). 23 compounds were detected in WD38 HTLAW, including 6 short-chain hydrocarbons, 9 heterocyclic compounds, and 8 aromatics. Table 2 displays the chromatograms of WD38 HTLAW after 6 days of fermentation with three *Rhodococcus* single strains (PD630, RHA1 and VanA⁻). Results showed that the total number of original components in WD38 HTLAW almost decreased by one third after 6 days of fermentation. Table 2 shows that microbial fermentation changed compositions and concentrations of the original HTLAW compounds. For example, *R. jostii* RHA1 decreased the total numbers of the original HTLAW compounds from 23 to 19, including 5 short-chain hydrocarbons, 8 heterocycle, and 6 aromatic compounds. After WD38 HTLAW was degraded by *R. jostii* RHA1 VanA⁻19, compounds were found in the degradation media, including 5 short-chain hydrocarbons, 8 heterocycle, and 6 aromatic compounds in fermentation broth. After incubation of *R. opacus* PD630 in WD38 HTLAW, the detected chemicals were reduced to 20 compounds including 6 short-chain hydrocarbons, 9 heterocycle and 5 aromatic compounds. In

the biodegradation of WD38 HTLAW, the aromatic compounds could be decreased to some extent with *R. opacus* PD630, *R. jostii* RHA1, and its mutant *R. jostii* RHA1 VanA⁻ single strain culture. 1,2-Benzenediol and its derivatives were not effectively degraded. Thus, it is necessary to employ an appropriate strategy for the effective biodegradation of HTLAW.

Moreover, Tet06 HTLAW contained 17 compounds, including 5 short-chain hydrocarbons, 10 heterocyclic compounds, and 2 aromatic compounds. This HTLAW was inoculated with single *Rhodococcus* strain culture and fermented for 6 days. Table 3 shows that the fermentation with *Rhodococcus* strains caused changes in the compositions and concentrations of compounds. It was found that *R. jostii* RHA1 decreased the total numbers of the compounds from 17 to 13, including 4 short-chain hydrocarbons, 8 heterocycle and 1 aromatic compound. After Tet06 HTLAW was degraded by *R. jostii* RHA1 VanA⁻, 16 compounds were detected, including 5 short-chain hydrocarbons, 9 heterocycle and 2 aromatic compounds. After the Tet06 HTLAW was degraded by *R. opacus* PD630, there were 17 compounds in the liquid phase, including 5 short-chain hydrocarbons, 11 heterocycle and 1 aromatic compound. Notably, this aromatic compound, 5-phenyl-2-pentanone, in Tet06 HTLAW could be biotransformed into lipids with *R. opacus* PD630, *R. jostii* RHA1, and its mutant *R. jostii* RHA1 VanA⁻ single culture while 3,5-dimethoxy phenol could be completely biodegraded by *R. opacus* PD630 and *R. jostii* RHA1.

As shown in Table 2&3, GC-MS analysis showed that new chemicals were generated through microbial action. These changes in compositions were different because WD38 HTLAW and Tet06 HTLAW had some complicated compounds to induce different degree of enzyme activity for the biodegradation of HTLAW. Thus, different metabolism pathways were followed when these three *Rhodococci* strains were used to degrade WD38 HTLAW and Tet06 HTLAW. Compared with Tet06 HTLAW, WD38 HTLAW as the more toxic diet might induce some

oxidases and/or other enzymes to degrade some toxic compounds. These results also indicated that all three bacteria strains could modify the components in HTLAW.

3.3. Biodegradation of chemical compounds in WD38 and Tet06 HTLAW by coculture with *R. opacus* PD630, *R. jostii* RHA, and its mutant *R. jostii* RHA1 VanA⁻

It was reported that *R. jostii* RHA1 and its ferredoxin oxygenase (VanA) deletion mutant strain *R. jostii* RHA1 VanA⁻ degraded lignin and lignin-derived aromatic compounds (Chen et al., 2012; Sainsbury et al., 2013). Additionally, *R. opacus* PD630 degraded some aromatic compounds via the β -ketoadipate pathway and accumulated lipids (Kosa and Ragauskas, 2012; Zhao et al., 2013). In this study, *Rhodococcus* consortium (*R. opacus* PD630, *R. jostii* RHA1, and its mutant *R. jostii* RHA1 VanA⁻) (hereafter referred to as V+P+R) was tested to degrade both WD38 and Tet 06 HTLAW. GC/MS results showed that the total number of detected original chemicals in the HTLAW decreased by over one third after coculture for 168 h. Higher degradation rates were obtained compared with single strain culture. As in Fig. 4a, 1,2-benzenediol, butanoic acid, butyrolactone, cyclopentanone, ethanol, phenol and propanoic acid in WD38 HTLAW were significantly reduced by 55.1%, 29.2%, 45.5%, 65.1%, 91.2%, and 76.2%, respectively. As shown in Fig. 4b, acetic acid, dianhydromannitol, 2-piperidinone, 2-methylpyrazine (pyrazine, methyl-), 2-pyrrolidinone, and 1-methyl-2-pyrrolidinone (2-pyrrolidinone, methyl-) in the Tet06 HTLAW stream were reduced by 43.4%, 15.6%, 55.2%, 66.2%, 21.5%, and 57.2%, respectively. With the coculture of V+P+R, 49.7% and 51.7% of COD reduction for WD38 and Tet06 HTLAW were obtained after 168 h, respectively (Data not shown). Compared with the single strain culture, lower COD reduction was obtained by coculture. However, effective biodegradations of aromatic compounds (1,2-benzenediol and phenol) were obtained by coculture of W38 HTLAW, and heterocycle compounds in Tet06

HTLAW could be significantly removed. Results suggested that coculture of these three strains could be used to degrade aromatics and heterocycle compounds, which might result in the high accumulation of lipid in cells. In coculture, the initial cellular lipid content in *R. opacus* PD630, *R. jostii* RHA1, and *R. jostii* RHA1 VanA was 0.099 g lipid/g CDW. 0.10 g lipid/L (0.080 g lipid/g CDW, 1.25 g CDW/L) and 0.46 g lipid/L (0.38 g lipid/g CDW, 1.21 g CDW/L) were incrementally accumulated during co-culture fermentation for 168 h in WD38 and Tet06 HTLAW, respectively (data not shown). Using ethanol organosolv lignin and its ultrasonicated product as carbon source (Kosa and Ragauskas, 2013), however, *R. opacus* DSM 1069 could accumulate up to 0.04 g lipid/g CDW. Based on the decreased COD and incremental accumulation of lipid, 0.0030 and 0.0162 mg lipid/mg O₂ were obtained from the biodegradation of WD38 and Tet06 HTLAW, respectively. Significantly, WD38 and Tet06 HTLAW could be well metabolized into lipids by coculture with *R. opacus* PD630, *R. jostii* RHA1, and its mutant *R. jostii* RHA1 VanA⁻.

4. Conclusion

Rhodococcus opacus PD630, *Rhodococcus jostii* RHA1, and its mutant *R. jostii* RHA1 VanA⁻ showed strong capacities in aromatics degradation and COD reduction. COD of WD38 and Tet06 HTLAW were decreased more than 38% by these strains. The total number of detected original chemicals in HTLAWs decreased by over 1/3 after the coculture of these strains for 168 h with 0.10 g/L and 0.46 g/L lipids accumulation in WD38 and Tet 06 HTLAW, respectively. Results highlight the fundamental knowledge of pathways and functional modules necessary for biological conversion of under-utilized carbon and toxic compounds in HTLAW into lipids for biofuels production.

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Figure and Table captions

Table 1. Characterization of HTLAW samples

Table 2. GC/MS chromatogram of WD38 HTLAW after six-days biological treatment with *Rhodococcus* strains (RHA1, VanA⁻, PD630)

Table 3. GC/MS chromatogram of Tet06 HTLAW after six-days biological treatment with *Rhodococcus* strains (RHA1, VanA⁻, PD630)

Fig. 1. COD (mg O₂/L) removal by *R. opacus* PD630, *R. jostii* RHA1, *R. jostii* RHA1 VanA⁻ single culture after 48 h of biodegradation

Fig. 2. Time courses for the removal of COD (mg O₂/L) of Tet06 HTLAW with *Rhodocuccuci*

Fig. 3. Effects of WD38 HTLAW (a) and Tet06HTLAW (b) on the cell growth and lipid accumulation

Fig. 4. The top 7 chemicals removal from WD38 HTLAW byproduct by a *Rhodococcus* consortium (coculture, 168 h) (a); Fig. 8. The top 6 chemicals removal from Tet06 HTLAW byproduct by a *Rhodococcus* consortium (coculture, 168 h) (b)

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Table 1. Characterization of HTLAW samples

Wastewater	Biomass resource	C	H	N	S	COD	pH
		(%) Wet basis	(%) Wet basis	(%) Wet basis	(%) Wet basis	(mg O₂/L)	
WD38 HTLAW	Pine wood	2.24	11.0	0.2	0	67,000	4.30
Tet06 HTLAW	Algae (<i>Tetraselmis</i>)	3.40	10.5	0.83	0.1	55,000	4.71

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3

1 Table 2. GC/MS chromatogram of WD38 HTLAW after six-days biological treatment with
 2 *Rhodococcus* strains (RHA1, VanA⁻, PD630)

Substances	WD38	RHA1	VanA ⁻	PD630
+	+	+	+
+	-	-	+
+	+	+	+
+	+	+	+
+	-	-	-
+	+	-	+
+	+	-	+
+	+	-	+
+	+	+	+

+	+	+	+
+	+	-	-
+	+	+	+
+	+	+	+
+	-	+	+
+	+	+	+
+	-	-	-
+	-	-	-
+	+	+	+

+	-	-	-
+	-	-	-
+	+	+	+
+	+	+	-
+	+	+	+
+	-	-	-
+	-	-	-
-	+	+	+
-	+	+	-

- + + +

- - + -

- - + +

- - + -

- - - +

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2

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2 Table 3. GC/MS chromatogram of Tet06 HTLAW after six-days biological treatment with
 3 *Rhodococcus* strains (RHA1, VanA⁻, PD630)

Substances	Tet06	RHA1	VanA-	PD630
+	+	-	-
+	+	+	+
+	-	-	-
+	-	-	+
+	-	-	+
+	+	+	+
+	+	+	+
+	-	-	-

+	+	+	+
+	+	+	+
+	+	+	+
+	-	-	-
+	+	+	+
+	-	-	-
+	+	+	+
+	-	-	-
+	-	-	-

+	-	-	-
+	-	-	-
+	-	+	-
-	+	+	-
-	+	+	-
-	+	-	+
-	-	+	-
-	-	+	-
-	+	+	+

-	-	+	-
-	-	+	-
-	-	-	+
-	-	-	+
-	-	-	+
-	-	-	+
-	-	-	+

1

2

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2
3 Fig. 1. COD (mg O₂/L) removal by *R. opacus* PD630, *R. jostii* RHA1, *R. jostii* RHA1 VanA⁻
4 single culture after 48 h of biodegradation. (Control: no bacteria was added in the HTLAW)

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Fig. 2. Time courses for the removal of COD (mg O₂/L) of Tet06 HTLAW with *Rhodocuccuci*

Fig. 3. Effects of WD38 HTLAW (a) and Tet06 HTLAW (b) on the cell growth and lipid accumulation

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2

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4 Fig. 4. The top 7 chemicals removal from WD38 HTLAW byproduct by a *Rhodococcus*
 5 consortium (co-culture, 168 h) (a); The top 6 chemicals removal from Tet06 HTLAW byproduct
 6 by a *Rhodococcus* consortium (coculture, 168 h) (b)

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