





# Genome Sequence of a *Byssochlamys* sp. Strain Isolated from Fouled B20 Biodiesel

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**ABSTRACT** *Byssochlamys* sp. strain AF001 is a filamentous fungus isolated from fouled B20 biodiesel. Its growth on B20 biodiesel results in the degradation and fouling of the fuel and higher rates of corrosion in affected storage tanks. The genome of *Byssochlamys* sp. AF001 is 35.9 Mbp and is composed of 10 scaffolds, with a G+C content of 45.89%.

Biodiesel is a widely used additive in ultralow-sulfur diesel (ULSD), both to improve the lubricating properties of the fuel and to reduce our reliance on fossil fuels (1). A 20% mixture of ULSD and biodiesel, B20, is commonly used across the United States. Unfortunately, biodiesel is more susceptible to degradation by microorganisms than is neat diesel (diesel without biodiesel added) (2). Studies into the microbial degradation of B20 have focused on either bacterial consortia or a common fungal fuel contaminant, *Hormoconis resinae* (3, 4). It is important to industry and end-users of biodiesel fuel blends that we expand our knowledge of other microorganisms capable of fuel degradation (5). A member of the filamentous fungal genus *Byssochlamys* (order Eurotiales; here referred to as *Byssochlamys* sp. AF001) was isolated from fouled B20 biodiesel in a storage tank on a U.S. Air Force base. The genome of *Byssochlamys* sp. AF001 was sequenced as a means to better understand its ability to grow on B20 as a sole carbon and energy source, to cause fouling and degradation of fuels, and to accelerate microbiologically influenced corrosion of carbon steel.

Genomic DNA was extracted from a pure culture of *Byssochlamys* sp. AF001 using the Xpedition soil/fecal DNA miniprep kit (Zymo Research Corp., Irvine, CA) for sequencing on the Illumina MiSeq platform using PE250 sequencing chemistry. Genomic DNA was also extracted for long-read sequencing to improve the assembly using an optimized phenol-chloroform-isoamyl alcohol method (6) and sequenced on an Oxford Nanopore MiniON sequencer using two R9 flow cells. Assembly was carried out with the SPAdes assembler version 3.11.1 (7), using k-mer values ranging from 21 to 127. Genome assembly statistics were computed using QUAST (8). An annotation was then produced using the GenSAS pipeline (<https://www.gensas.org/>) and GhostKOALA (9).

The *Byssochlamys* sp. AF001 assembly was composed of 10 scaffolded contiguous sequences. The  $N_{50}$  was 4.6 Mbp, the total genome length was 35.9 Mbp, and the G+C content was 45.89%. A putative esterase/lipase, potentially able to cleave the fatty acid-methyl ester (FAME) to its constituent fatty acid and methanol, was located on scaffold 4. Analysis by GhostKOALA suggests that *Byssochlamys* sp. AF001 is capable of metabolizing methanol during the degradation of FAME found in biodiesel, similar to other fungi (10).

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The genome described here is of particular interest to further studies of biodiesel degradation both in field and laboratory experiments. Future work will leverage this genome sequence and others to better understand the underlying molecular mechanisms of biodiesel degradation.

**Accession number(s).** This whole-genome shotgun sequencing project has been deposited at GenBank under the accession number [PNEM00000000](https://www.ncbi.nlm.nih.gov/nuclseq/PNEM00000000).

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