ORIGINAL ARTICLE

Biopretreatment of palm oil mill effluent by thermotolerant polymer-producing fungi

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Abstract

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Biopretreatment of palm oil mill effluent by thermotolerant polymer-producing fungi

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Palm oil industry is one of the three major agro-industries in Southern Thailand and generates large quantities of effluent with high organic matter (BOD and COD values of 58,000 and 110,000 mg/l, respectively), total solids and suspended solids (70,000 and 40,000 mg/l, respectively), oil & grease (25,600 mg/l), and has a low pH (4.5). Conventional anaerobic ponding system is normally employed in palm oil mills to treat the effluent. To increase its efficiency, biopretreatment to remove the organic matter and oil & grease by thermotolerant polymer-producing fungi was investigated. The palm oil mill effluent (POME) was treated by the two thermotolerant polymer-producing fungi, *Rhizopus* sp. ST4 and *Rhizopus* sp. ST29, at 45ºC under aseptic and septic conditions. *Rhizopus* sp. ST4 gave the same oil & grease removal (84.2%) under both conditions but COD removal under septic condition (62.2%) was 8.8% higher than that under aseptic condition (53.4%). On the contrary, *Rhizopus* sp. ST 29 under aseptic condition showed 11% and 25.4% higher oil & grease removal (91.4%) and COD removal (66.0%) than those under septic condition. Comparison between the two isolates under aseptic condition revealed that *Rhizopus* sp. ST29 exhibited higher oil & grease removal

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(91.4%) as well as COD removal (66.0%) than those of *Rhizopus* sp. ST4 (84.2% and 53.4%, respectively). Under septic condition, *Rhizopus* sp. ST4 gave higher oil & grease removal (84.2%) and COD removal (62.2%) than did *Rhizopus* sp. ST 29 (80.5 and 40.6%, respectively).

Key words : palm oil mill effluent, biopretreatment, thermotolerant fungi, polymer

บทคัดย่อ

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การบำบัดขั้นต้นแบบชีวภาพของน้ำทิ้งโรงงานสกัดน้ำมันปาล์มโดยใช้เชื้อราทนร้อนที่ผลิตพอลิเมอร์

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อุตสาหกรรมน้ำมันปาล์มเป็นหนึ่งในสามของอุตสาหกรรมเกษตรหลักในภาคใต้ของประเทศ น้ำทิ้งจากโรงงานสกัดน้ำมันปาล์มประกอบด้วยสารอินทรีย์ (ค่าบีโอดี และซีโอดี เท่ากับ 58,000 และ 110,000 มก./ลิตร ตามลำดับ), ของแข็งทั้งหมด และของแข็งแขวนลอย (70,000 และ 40,000 มก./ลิตร ตามลำดับ), น้ำมันและกรีส (25,600 มก./ลิตร) ในปริมาณสูงและพีเอชต่ำ (4.5) ระบบบำบัดน้ำเสียที่โรงงานสกัดน้ำมันปาล์มนิยมใช้คือระบบบ่อไร้อากาศแบบธรรมดาและเพื่อเพิ่มประสิทธิภาพการบำบัดของระบบจึงศึกษาการบำบัดขั้นต้นแบบชีวภาพเพื่อกำจัดสารอินทรีย์ น้ำมันและกรีส จากการบำบัดน้ำทิ้งโรงงานสกัดน้ำมันปาล์มโดยใช้เชื้อราทนร้อนที่ผลิตพอลิเมอร์จำนวน 2 สายพันธุ์ คือ *Rhizopus* sp. ST4 และ *Rhizopus* sp. ST29 ที่อุณหภูมิ 45°C ภายใต้สภาวะปลอดเชื้อ และไม่ปลอดเชื้อ พบว่า *Rhizopus* sp. ST4 สามารถกำจัดน้ำมันและกรีสได้เท่ากัน (84.2%) ทั้งสองสภาวะ แต่ภายใต้สภาวะไม่ปลอดเชื้อสามารถกำจัดซีโอดี (62.2%) ได้สูงกว่าสภาวะไม่ปลอดเชื้อ (53.4%) เท่ากับ 8.8% สำหรับ *Rhizopus* sp. ST 29 สภาวะปลอดเชื้อให้ค่าการกำจัดน้ำมันและกรีส (91.4%) และค่าการกำจัดซีโอดี (66.0%) สูงกว่าค่าที่ได้จากการเลี้ยงเชื้อภายใต้สภาวะไม่ปลอดเชื้อ (11% และ 25.4% ตามลำดับ) เมื่อเปรียบเทียบระหว่างสองสายพันธุ์ภายใต้สภาวะปลอดเชื้อ พบว่า *Rhizopus* sp. ST29 ให้ค่าการกำจัดน้ำมันและกรีส (91.4%) และค่าการกำจัดซีโอดี (66.0%) สูงกว่าสายพันธุ์ ST4 (84.2% และ 53.4% ตามลำดับ) ส่วนการบำบัดภายใต้สภาวะไม่ปลอดเชื้อ *Rhizopus* sp. ST4 ให้ค่าการกำจัดน้ำมันและกรีส (84.2%) และค่าการกำจัดซีโอดี (62.2%) สูงกว่าค่าที่ได้จาก *Rhizopus* sp. ST29 (80.5% และ 40.6% ตามลำดับ)

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Palm oil mill effluent (POME) is the mixed effluent generated from two major sources; sterilizer and decanter or separator during the extraction of palm oil. The average quantity of POME in Thailand was found to be 0.87 m^3 per ton of fresh fruit bunch (H-Kittitun *et al*., 1994). POME usually contained very high organic matter, suspended solids and oil (1-2%) (Prasertsan *et al*., 1990). Biological treatment is a high efficiency method to eliminate organic matter, suspended solids and oil. Treatment of palm oil mill effluent using micro-organisms, such as some fungi, yeast and photo-synthetic bacteria (Baker and Worgan, 1981; Muneesri, 1995) or using enzymes (commercial

enzymes and enzymes extracted from microorganisms) (Chantapaso, 1999; Muneesri, 1995), has been previously studied. This work aims to investigate pretreatment of the POME using thermotolerant polymer-producing fungi to remove organic matter and oil & grease.

Material and Methods

Microorganisms

Two thermotolerant polymer-producing fungal strains, *Rhizopus* sp. ST4 and *Rhizopus* sp. ST29, were isolated from Thaksin Palm Oil Industy at Surat Thani Province and were previously studied by Muneesri (1995).

Characteristics of POME

Decanter effluent was taken from Pure Oil Co., Ltd., Hat Yai. The samples were analyzed for chemical oxygen demand (COD), biological oxygen demand (BOD), total solids (TS) and suspended solids (SS), total alkalinity, total acidity, pH, oil & grease (APHA, AWWA, and WEF, 1998) and total nitrogen (A.O.A.C., 1984).

Biopretreament of POME under septic and aseptic condition

Spore suspension preparation: Spore suspension was prepared by adding 10 ml of 0.1% Tween 80 onto PDA slant of 5 days old culture of *Rhizopus* sp. ST4 and *Rhizopus* sp. ST29 and the concentration was adjusted to reach 2.4×10^6 spores/ml.

Cultivation: Starter culture (10% v/v) of each thermotolerant fungal isolate, *Rhizopus* sp. ST4 and *Rhizopus* sp. ST29 was inoculated into 500 ml Erlenmeyer flasks containing 100 ml each of septic POME (POME not autoclaved) and aseptic POME (POME autoclaved at 121ºC for 15 min). They were incubated at 45ºC on a shaker (200 rpm). Samples were taken every 24 h for 4 days to determine pH, COD, and oil & grease. Statistical analysis was conducted with SPSS program by Duncan's model at 0.05 level.

Results and Discussion

Characteristics of POME

Palm oil mill effluent is mainly generated from sterilization and clarification processes in which large amounts of steam and/or hot water are used (Ma, 1990) Characteristics of the decanter effluent taken from a palm oil mill were determined and found to contain high organic matter (BOD and COD values of 58,475 mg/l and 110,400 mg/l, respectively) high total solids and suspended solids (71,930 mg/l and 43,280 mg/l respectively), high oil & grease (25,600 mg/l), and low nitrogen content (900 mg/l), and to have low pH (4.5) (Table 1). The results were similar to those of previous studies (Chin and Wong, 1983; Muneesri, 1995; Ng *et al*., 1985; Prasertsan *et al*., 1990). The high organic matter is due to the presence of different sugars such as arabinose, xylose, glucose, galactose and manose at the concentrations of 6.43, 0.44, 0.22, 0.15 and 0.10% dry weight, respectively (Agamutha and Tan, 1985). The oil residue was in the range of 1-2% which depends very much on the quality of raw material (palm fruits), process control and machine efficiency. The suspended solids in the POME are mainly oil-bearing cellulosic materials from the fruits (Ma, 1990). Since the POME is non-toxic as no chemical is added in the oil extraction process, it is a good source of nutrients for microorganisms.

Table 1 Characteristics of palm oil mill effluent

Parameter	Range	Mean
pH	4.2-4.8	4.5
BOD (mg/l)	43,300-89,000	58,500
COD (mg/l)	90,000-179,000	110,000
Total solids (mg/l)	51,200-105,000	71,900
Suspended solids (mg/l)	24,300-76,800	43,300
Total alkalinity (mg/l)	240-1,240	700
Total acidity (mg/l)	612-3,500	2,740
Oil & grease (mg/l)	5,700-57,500	25,600
Total nitrogen (mg/l)	750-1000	900

Effect of biopretreatment substrate

Due to the high concentrations of organic matter and oil & grease in POME, biological pretreatment using the thermotolerant fungal isolates was tested under septic and aseptic conditions. Pretreatment of POME by *Rhizopus* sp. ST 4 under aseptic aerobic (200 rpm) condition at 45ºC could reduce the organic matter by half (48.4% COD removal) within 2 days with only slight further removal to 53.4% and 54.9% after 3 and 4 days, respectively (Figure 1). This was due to the uptake of organic matter as a carbon source by the microorganism. Oil & grease removal was higher than the COD removal and increased with the increased cultivation time, giving the values of 73.1%, 80.4%, 84.2% and 91.7% after 1, 2, 3, and 4 days, respectively. Consideration of both results indicated that 3 days cultivation was optimal for POME pretreatment as it gave COD removal not significantly different from that of 4 days (p=0.05) and very high oil & grease removal.

Under aseptic condition, pretreatment of POME by *Rhizopus* sp. ST 29 could reduce organic matter higher than *Rhizopus* sp. ST4 at the same condition. The COD removals were 61.5%, 66.0% and 69.2% after 2, 3, and 4 days incubation (Figure 1). These values were similar to previous studies which gave the values of 62.1% and 69.5% after 3 and 4 days incubation, respectively (Muneesri, 1995). Oil & grease removal were 79.3%, 91.4%, and 91.8% after 2, 3 and 4 days incubation respectively. COD removal at day 3 was 11% higher than at day 2 but not significantly different from that at day 4 (at p=0.05). *Rhizopus* sp. ST29 could uptake oil & grease as a carbon source as well as the *Rhizopus* sp. ST4. Oil & grease was in emulsion form as it dissolved at 45ºC. Comparison of the performance of the two strains under aseptic aerobic condition indicated that *Rhizopus* sp. ST 29 gave 13.0% higher COD removal and 8% higher oil & grease removal after 3 days cultivation. Oil & grease could also be removed by enzymatic method using the commercial xylanase (Meicellase) and enzyme extracted from *Aspergillus niger* ATCC 6275 and incubated at 40ºC as it gave more than 99% oil & grease removal and COD removals of 76% and 69.4%, respectively (Chantaphaso, 1999). Besides, the enzymes were able to separate sus-

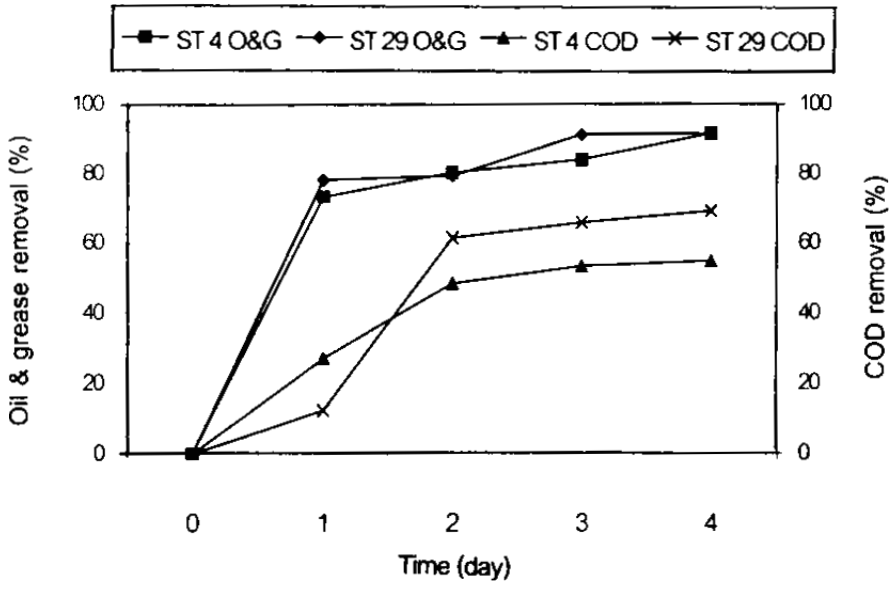

Figure 1 COD and oil & grease removal of POME treated by *Rhizopus* sp. ST 4 and *Rhizopus* sp. ST 29 with aseptic condition at 45ºC and shaker speed of 200 rpm

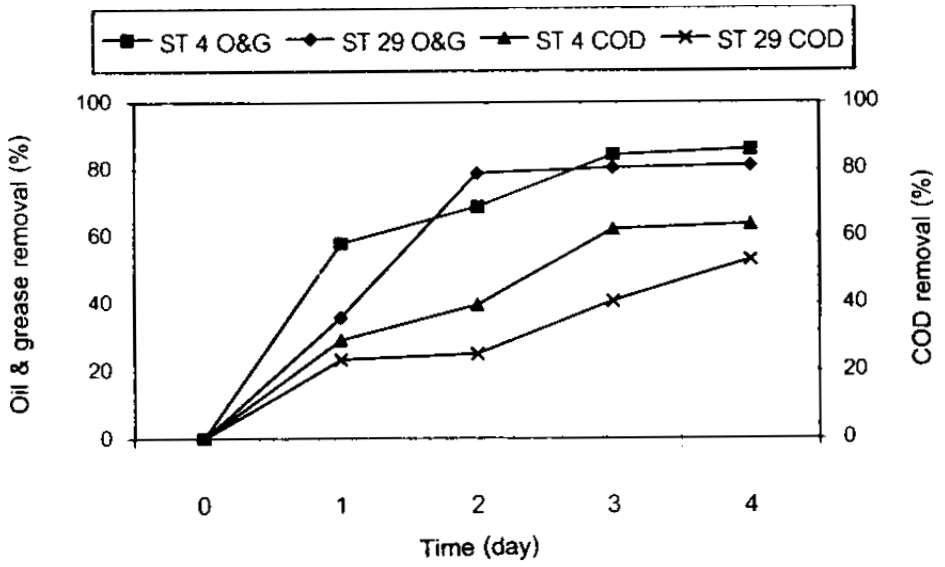

Figure 2 COD and oil & grease removal of POME treated by *Rhizopus* sp. ST 4 and *Rhizopus* sp. ST 29 with septic condition at 45ºC and shaker speed of 200 rpm

pended solids which floating as bulking solids at 70.6 and 69.8%, respectively (Maneesri, 1995).

Under septic condition (Figure 2), the thermotolerant fungal *Rhizopus* sp. ST 4 gave 62.2% COD removal and 84.2% oil & grease removal after 3 days incubation. These values were not significantly different from those of 4 days cultivation. *Rhizopus* sp. ST 29, on the other hand, gave 40.6% COD removal with the oil & grease removal of 80.5% after 3 days cultivation. While COD removal still increased significantly to 53.1% at day 4, the oil & grease removal increased very slightly to 81.3%. Comparison on the pretreatment of POME by the two strains under septic condition indicated that *Rhizopus* sp. ST 4 gave 21.6% higher COD removal and 3.7% higher oil & grease removal, after 3 days cultivation, than *Rhizopus* sp. ST 29.

Performance of the isolate on pretreatment of POME by the two thermotolerant fungal isolates is summarized in Table 2. After 3 days cultivation *Rhizopus* sp. ST 4 gave higher COD removal under septic condition than under aseptic condition with the same oil & grease removal. This is contrary to the results obtained from *Rhizopus* sp. ST 29, which performed better under aseptic condition.

Table 2 Comparison of palm oil mill effluent by *Rhizopus* sp. ST 4 and *Rhizopus* sp. ST 29 at 45ºC on a shaker (200 rpm) after 3 days cultivation

Strain	condition	COD removal (%)	Oil & grease removal (%)
ST 4	Aseptic	53.4[b]	84.2[a]
ST 4	Septic	62.2[c]	84.2[a]
ST 29	Aseptic	66.0[d]	91.4[b]
ST 29	Septic	40.6[a]	80.5[a]

Remark: average value from 2 experiments, a, b, c, d means in the same row with different superscripts are significantly different (p=0.05)

Under septic condition, the decrease of COD or oil & grease was the result of the metabolism of the organic substances by each thermotolerant fungal isolate together with the other trophics originally present in the POME. Under aseptic condition, on the other hand, COD and oil & grease removals were derived only from the efficiency of each strain, *Rhizopus* sp. ST 4 or *Rhizopus* sp. ST 29. COD and oil & grease removals under aseptic condition were higher than those under septic condition for *Rhizopus* sp. ST 29. The COD removal of *Rhizopus* sp. ST 4 under septic condition was higher than that under aseptic condition with no difference in oil & grease removal. However, oil & grease removal was higher than COD removal in all conditions which may due to the preference of the fungi to uptake oil & grease as a carbon source rather than other sources. The digestion of other organic matter (COD removal) took longer as POME was low in nitrogen content. It was found that oil & grease was the main component of organic matters in POME as it reduced up to 80%-90% while the COD removal decreased by 50-70%. The optimal condition for POME biopretreatment by *Rhizopus* sp. ST 4 and *Rhizopus* sp. ST29 were septic and aseptic condition, respectively, at 45ºC on a shaker (200 rpm). From practical point of view, septic condition was more favorable than aseptic condition as it was more economical and consumed less power as autoclaving the POME was not required.

Conclusion

Palm oil mill effluent (POME) could be efficiently pretreated at high temperature (45ºC) by *Rhizopus* sp. ST4 and *Rhizopus* sp. ST29 under septic and aseptic condition, respectively. Under septic condition, *Rhizopus* sp. ST4 could remove 84.2% oil & grease and 62.2% COD, whereas under aseptic condition, *Rhizopus* sp. ST29 gave the values of 91.4% and 66.0%, respectively, within 3 days cultivation.

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