Biodiesel and ethanol from salt-tolerant seashore mallow

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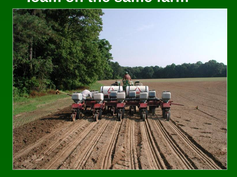
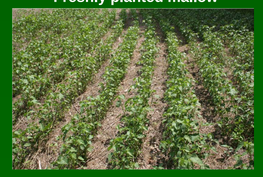
No-till planting in Sussex County, Delaware

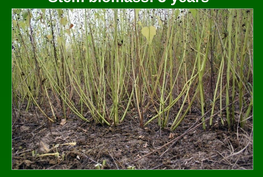
Planting in tilled sandy loam on the same farm



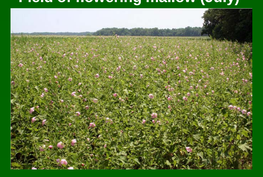
Freshly planted mallow



Stem biomass: 3 years



Field of flowering mallow (July)

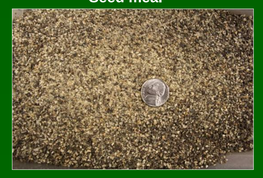


Flower

Harvesting mallow (September)

Seeds

Seed meal



INTRODUCTION

General features of seashore mallow:

- *Kosteletzkya pentacarpos* (L.) Ledebour
- Halophytic dicot of the Malvaceae family
- Also known as Virginia saltmarsh mallow
- Native to the Gulf and Atlantic coasts of the U.S.
- Perennial (10-12 years) and reaches maturity in 3 years
- Grows to 0.6-1.3 m in height
- Produces large pink blooms (2.5-5 cm) in July/August
- Salt-tolerant, drought-tolerant and waterlogging-tolerant
- Seeds do not readily shatter
- No known diseases and not invasive
- Planted and harvested with traditional farm equipment
- Stores carbon below ground in large perennating root
- Can be irrigated with brackish or seawater

How to grow and harvest seashore mallow:

- 1st year: produces one stem that can be harvested in fall
- Stems increase in subsequent years to 12+
- Root crown lives up to 10-12 years

Biodiesel and ethanol from seashore mallow:

- Seeds contain high protein (32%) and oil (22%) content
- Yield of oil is 330 kg/ha (soybean oil: 350-560 kg/ha)
- After maturity, produces up to 44 woody stems annually
- Stems contain 518 g/kg of carbohydrates
- Theoretical maximum yield of ethanol: 0.38 L/kg

Big picture: Seashore mallow can use resources not usable by food crops (saline land and water), thus not displacing food production while simultaneously enhancing biofuels production.

OBJECTIVES

Overall:

- Determine feasibility of biodiesel and cellulosic ethanol production from seashore mallow

Biodiesel:

- Prepare fatty acid methyl esters from seashore mallow oil
- Measure fuel properties of methyl esters
- Blend methyl esters with petrodiesel and measure properties
- Compare results with fuel standards

Ethanol:

- Measure composition of residual seed meal and stem biomass to determine potential ethanol yield
- Pretreat biomass to liberate cellulose from lignin
- Enzymatic digestion to decompose cellulose into sugars
- Ferment sugars to ethanol using *Saccharomyces cerevisiae*

ACKNOWLEDGEMENTS

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BIODIESEL RESULTS

Properties of seashore mallow oil methyl esters (SMME) with a comparison to biodiesel standards ASTM D6751 and EN 14214. Also depicted are test methods utilized in the current study along with the number of trials performed for each determination.

Specification	Abbrev.	Units	ASTM D6751	EN 14214	Test Method	Trials	SMME
Acid value	AV	mg KOH/g	0.50 max	0.50 max	AOCS Cd 3d-63	3	0.13 ± 0.01
Free glycerol	FG	mass %	0.020 max	0.020 max	ASTM D6584	1	0.009
Total glycerol	TG	mass %	0.240 max	0.250 max	ASTM D6584	1	0.068
Moisture	-	ppm	-	500 max	ASTM D6304	3	373 ± 5
Kinematic viscosity, 40 ºC	KV	mm^2/s	1.9-6.0	3.5-5.0	ASTM D445	3	4.44 ± 0.01
Induction period, 110 ºC	IP	h	3.0 min	6.0 min	EN 15751	3	1.7 ± 0.1
Cloud point	CP	ºC	Report	-	ASTM D5773	3	4.2 ± 0.5
Cold filter plugging point	CFPP	ºC	-	Variable	ASTM D6371	3	4.0 ± 0.6
Pour point	PP	ºC	-	-	ASTM D5949	3	3.7 ± 0.6
Derived cetane number	DCN	-	47 min	51 min	ASTM D6890	32	59.9 ± 1.6
Iodine value	IV	g I_2/100 g	-	120 max	AOCS Cd 1c-85	1	111
Sulfur	S	ppm	15 max	10 max	ASTM D5443	3	2
Phosphorous	P	mass %	0.001 max	0.001 max	ASTM D4951	3	none detected
Wear scar, 60 ºC	–	µm	-	-	ASTM D6079	3	155 ± 8
Energy content	HHV	MJ/kg	-	-	ASTM D4809	3	39.53 ± 0.13
Specific gravity, 15.6 ºC	SG	-	-	-	AOCS Cc 10c-95	3	0.887 ± 0.001
Gardner color	-	-	-	-	AOCS Td 1a-64	1	11

Properties of SMME blended with ultra-low sulfur (< 15 ppm) diesel (ULSD) fuel with a comparison to petrodiesel standards ASTM D975, EN 590 and ASTM D7467.

Specification	ASTM D975	ASTM D7467	EN 590	ULSD	B5	B20
Biodiesel content, vol %	0 - 5	6 - 20	0 – 7	0	5.0	20.0
Acid value	-	0.30 max	-	none	none	0.15 ± 0.02
Moisture	-	-	200 max	17 ± 1	60 ± 2	159 ± 1
Kinematic viscosity, 40 ºC	1.9 – 4.1	1.9 – 4.1	2.0 – 4.0	2.30 ± 0.01	2.31 ± 0.01	2.49 ± 0.01
Induction period, 110 ºC	-	6.0 min	20.0 min	40.1 ± 1.5	7.0 ± 0.1	3.6 ± 0.2
Cloud point	-	-	-	-17.5 ± 0.1	-13.6 ± 0.1	-11.3 ± 0.2
Cold filter plugging point	-	-	-	-18.0 ± 0	-17.0 ± 0	-16.7 ± 0.6
Pour point	-	-	-	-24.0 ± 0	-23.7 ± 0.6	-16.7 ± 0.6
Sulfur	15 max	15 max	10 max	9	8	7
Wear scar, 60 ºC	520 max	520 max	460 max	571 ± 4	296 ± 22	207 ± 9
Energy content	-	-	-	46.2 ± 0.1	45.2 ± 0.3	43.8 ± 0.6
Specific gravity, 15.6 ºC	-	-	-	0.837 ± 0	0.850 ± 0	0.855 ± 0

Fatty acid composition (area %) of crude seashore mallow oil.

Area % (n=3)	SMME
C14:0	0.2 ± 0
C16:0	24.4 ± 0.2
C16:1 c9	0.8 ± 0.1
18:0	2.2 ± 0.1
18:1 c9	18.3 ± 0.1
18:2 c9, 12	48.9 ± 0.2
18:3 c9, 12, 15	3.3 ± 0.1
C20:0	0.6 ± 0
C20:1 11c	1.2 ± 0.1
Total saturated	27.4
Total monounsaturated	20.3
Total polyunsaturated	52.2

Improvement in oxidative stability of blends of SMME with ULSD upon addition of 1000 ppm BHT.

EN 15751	B5	B20	B100
IP, 110 ºC	7.0 ± 0.1	3.6 ± 0.2	1.7 ± 0.1
With 1000 ppm BHT	57.5 ± 5.1	22.7 ± 1.8	4.6 ± 0.1

Preparation of biodiesel:

AV of crude oil was lowered from 2.72 to 0.08 mg KOH/g with pretreatment (1.0 vol % H_2SO_4; 35 vol % methanol; reflux; 4 h.

Acid-pretreated oil (250 g) and methanol (6:1 mole ratio) were combined and heated to 60 ºC, followed by addition of sodium methoxide catalyst (0.50 wt %). After stirring for 1 h, contents were cooled to room temperature, glycerol was removed, methanol was removed by rotary evaporation, and the crude product was washed until neutral pH and dried with $MgSO_4$. SMME was obtained in 94% yield.

ETHANOL RESULTS

Chemical composition of seashore mallow seed meal and stems (g/kg, dry basis). Stems were selected for further investigation due to their higher potential ethanol yield. Blast and core cells accounted for 40.8% and 59.2% of whole material.

Component (g/kg, dry)	Seed Meal	Stems	Blast Cells	Core Cells
Extractable	9.0 ± 0	14.0 ± 0.1	N/A	N/A
Protein	254.0 ± 8.5	61.5 ± 0.7	N/A	N/A
Total carbohydrates	438	518	545	565
Glucan	214 ± 10	337 ± 6	368 ± 16	361 ± 2
Xylan	145 ± 19	158 ± 3	147 ± 6	187 ± 1
Arabinan	16.7 ± 7.4	11.0 ± 2.7	17.8 ± 4.0	5.8 ± 1.5
Galactan	72.2 ± 14.4	11.7 ± 1.5	12.3 ± 3.3	12.1 ± 0.5
Lignin	246 ± 10	183 ± 4	146 ± 3	264 ± 14
Ash	62.0	93.2	N/A	N/A
Total g/kg accounted for	1000	856	N/A	N/A
Potential ethanol (L/kg)	0.33	0.38	0.40	0.41

Distribution of seashore mallow (http://plants.usda.gov)

Enzymatic digestion results following various pretreatments of ground seashore mallow stems. Dilute ammonium hydroxide provided the highest yield of sugars.

Pretreatment (n=3)	Conditions	Efficiency (% max)		Yields (g/kg, db)	
		Glucose	Xylose	Glucose	Xylose
Hydrothermal	180 ºC, 20 min	21 ± 0.7	31 ± 0.6	79 ± 2.7	55 ± 0.9
	200 ºC, 20 min	39 ± 4.2	32 ± 0.4	145 ± 16	58 ± 0.7
Ammonium hydroxide	180 ºC, 20 min	46 ± 3.0	46 ± 1.1	173 ± 11	77 ± 6.5
	200 ºC, 20 min	50 ± 1.2	50 ± 1.1	189 ± 4.3	74 ± 1.7
2% (w/v) H_2SO_4	180 ºC, 20 min	44 ± 1.1	44 ± 2.1	165 ± 7.1	56 ± 2.0
Alkaline H_2O_2	35 ºC, 24 h	15 ± 0.6	15 ± 1.1	55 ± 2.0	43 ± 3.0

Fermentation results for ammonium hydroxide-pretreated whole and fractionated seashore mallow stems. Simultaneous saccharification and fermentation was conducted employing a native Saccharomyces cerevisiae yeast strain.

Fermentation	Concentration (g/L)			Yields (g/kg, db)		Efficiency (% max)	
	Ethanol	Xylose	Glycerol	Ethanol	Xylose	Ethanol	Xylose
Whole	14.7 ± 1.2	6.8 ± 0.5	0.9 ± 0.1	104 ± 7.8	47.8 ± 1.6	54.4 ± 4.1	27.0 ± 0.9
Outer layer	19.5 ± 1.1	6.1 ± 0.2	0.7 ± 0	144 ± 5.7	45.2 ± 1.3	69.2 ± 2.7	27.0 ± 0.8
Core	1.5 ± 0.4	5.3 ± 0.5	0.2 ± 0	15.7 ± 5.1	55.6 ± 1.2	7.7 ± 2.4	26.2 ± 0.6

CONCLUSIONS

Biodiesel was prepared in high yield by transesterification of hexane-extracted SMO. The crude oil was initially treated with catalytic H_2SO_4 and methanol to lower its AV from 2.72 to 0.08 mg KOH/g, thus rendering it amenable to sodium methoxide-catalyzed methanolysis. The principal components identified in SMME were methyl linoleate (48.9%), methyl palmitate (24.4%) and methyl oleate (18.3%). The fuel properties of SMME were within the specifications listed in ASTM D6751 and EN 14214 with the exception of IP, which was improved upon addition of 1000 ppm BHT. Of note was the high DCN (59.9) of SMME along with its low IV (111 g I_2/100 g), which both represent advantages over FAMEs prepared from soybean oil. The properties of SMME blended with ULSD were within the ranges specified in the petrodiesel standards after the addition of BHT. Lastly, the suitability of residual seashore mallow biomass (stems) as a bioethanol fermentation feedstock was determined to have ethanol and xylose yields of 104 g/kg and 47.8 g/kg, respectively. Of the four pretreatment methodologies explored, dilute NH_4OH provided the highest yield of sugars.