ENTERIC BACTERIA AND BIODIESEL GLYCEROL BYPRODUCT IN UNDERGRADUATE RESEARCH

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ABSTRACT

Enteric Bacteria and Biodiesel Glycerol Byproduct in Undergraduate Research
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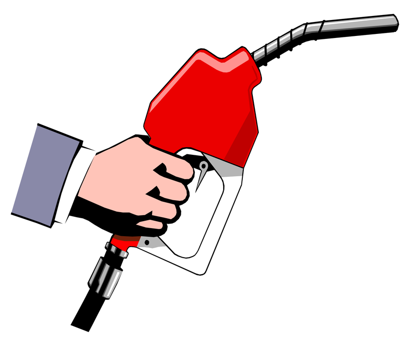
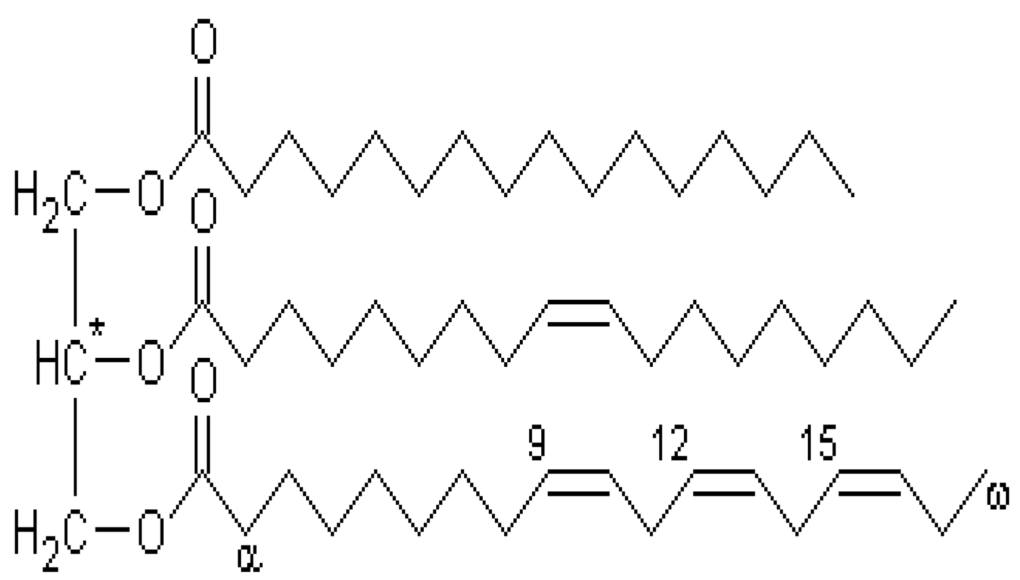
Escherichia coli and *Klebsiella* species have become important in biofuel research because strains have been engineered that produce ethanol, butanol or microdiesel. These bacteria which naturally utilize pure glycerol were assessed for potential to utilize biodiesel glycerol byproduct made by transesterification or catalyst column. To determine if byproduct contained inhibitory components *E. coli* was cultured in nutrient broth to which byproduct had been added. Culture optical density (OD) was measured by use of a Klett – Summerson colorimeter. Growth in culture containing byproduct from transesterification was slightly decreased while that in culture containing byproduct from catalyst column was increased. Bacteria were grown in minimal broth to evaluate byproduct as an individual carbon source. Byproduct made by transesterification was pH adjusted due to high alkalinity. There was not a considerable difference between culture ODs produced from glycerol and byproduct from catalyst column. Byproduct from transesterification did not support growth as well as that from catalyst column. *K. pneumoniae* appeared to utilize glycerol and byproduct better than *E. coli*. Both organisms grew significantly better on glucose than glycerol. A rapid solid phase glucose oxidase and peroxidase based assay (Precision Labs, Inc.) was used to detect glucose in cultures. Both *E. coli* and *K. pneumoniae* lowered added glucose to an undetectable level. A rapid solid phase alcohol oxidase and peroxidase based assay (AlcoScreen) was used to detect alcohol. *E. coli* showed a positive test for the presence of alcohol in cultures produced from glucose or glycerol. It was unknown if alcohol was made from byproduct because stock solutions showed a positive test for the presence of alcohol. These observations indicate that enteric bacteria may be able to utilize biodiesel glycerol byproduct as an industrial feedstock.

BACKGROUND



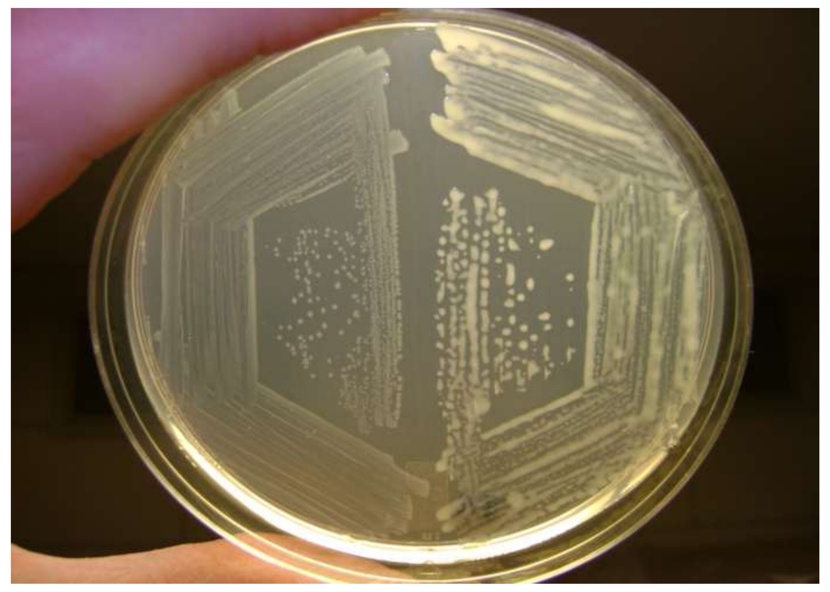
- Biodiesel is made by a process of alkaline hydrolysis of biological oil by using methanol, sodium hydroxide and heat. One liter of biodiesel glycerol byproduct is made for every three liters of biodiesel. Some laboratories are investigating the use of catalyst in a column to break oil into biodiesel and glycerol.
- *Escherichia coli* and *Klebsiella* species have become important in biofuel research because strains have been constructed by genetic engineering that produce ethanol (Ohta et al., 1991). *E. coli* has recently been genetically engineered to produce butanol and microdiesel respectively (Connor and Liao, 2008; Kalscheuer et al., 2006). These bacteria are known to naturally utilize pure glycerol as a carbon source.
- Glycerol is not a biofuel but it may be possible for some microorganisms that utilize glycerol byproduct to make new products as well as help solve a waste disposal problem.
- In this study, undergraduate students assessed growth of *E. coli* and *K. pneumoniae* in rich and minimal media with glycerol byproduct made by transesterification or catalyst column as a carbon source.



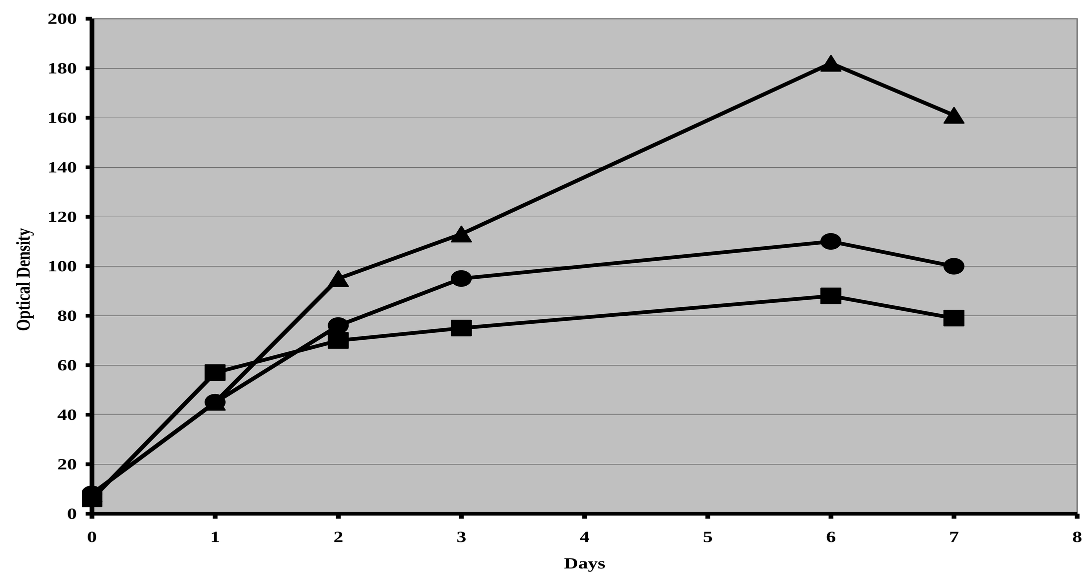
Fat triglyceride shorthand formula example (biological oil).
Left, glycerol.
Right, fatty acids.
Chemical formula, $C_{55}H_{98}O_6$.

METHODS AND RESULTS

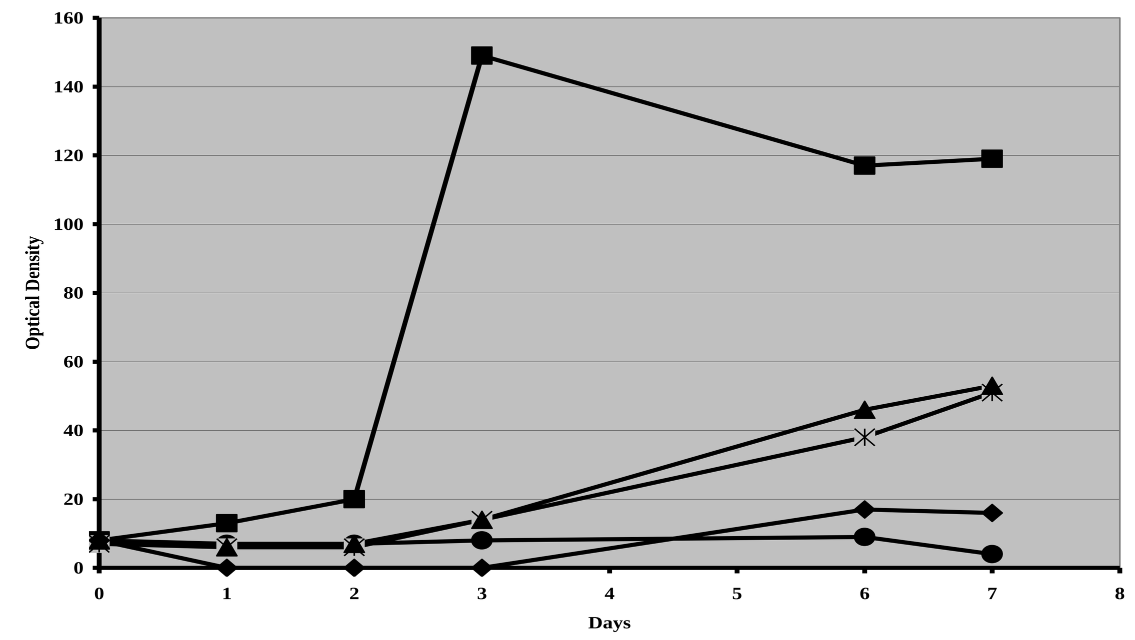
Stock solutions of glycerol byproduct from manufacture of biodiesel.
Left, glycerol byproduct from catalyst column (Next Energy).
Right, glycerol byproduct from transesterification (Power Alternative).
Concentration, 20%.



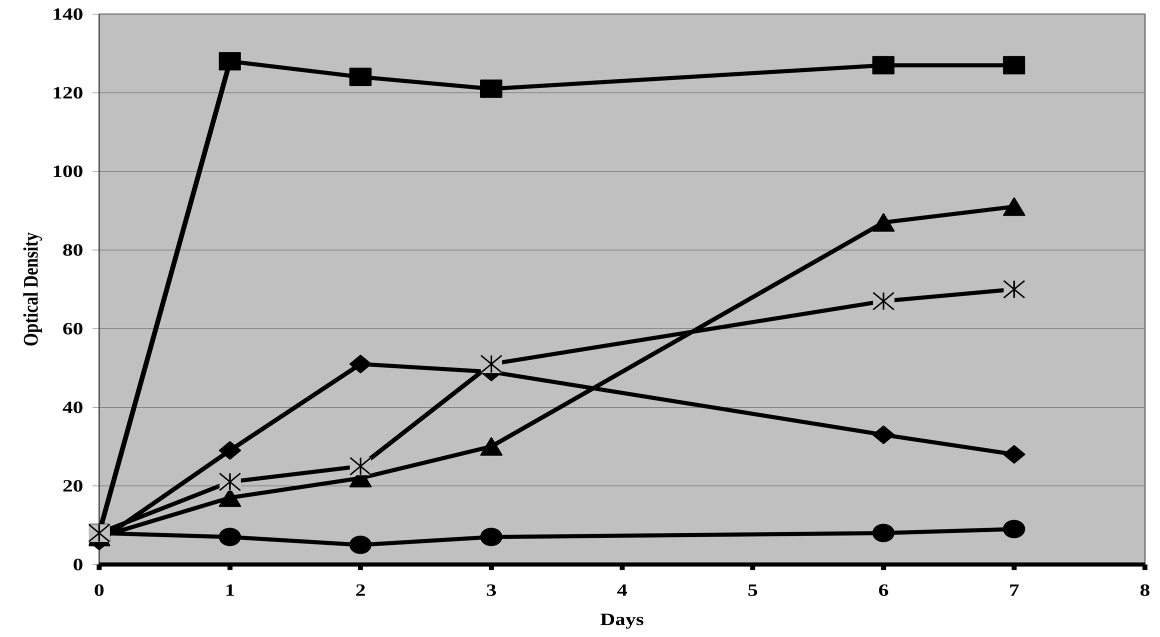
Growth of microorganisms on nutrient agar.
Left, *E. coli* K12.
Right, *K. pneumoniae*.
Incubation at 37°C for 1 day.



Growth of *E. coli* K12 in nutrient broth containing glycerol byproduct. Circle, no byproduct added; square, byproduct from transesterification; triangle, byproduct from column.
Concentration of sterilized byproduct added was .2%.
Byproduct was not pH adjusted.
Inoculum cells grown on nutrient agar.
Incubation at 25°C.



Growth of *E. coli* K12 in minimal broth containing glycerol byproduct.
Circle, no carbon source; square, glucose; triangle, glycerol; diamond, byproduct from transesterification; star, byproduct from column.
Minimal broth, Miller (1972). Sodium chloride substituted for sodium citrate.
Concentration of carbon source was .2%.
Byproduct was pH adjusted and sterilized.
Incubation at 25°C.



Growth of *K. pneumoniae* in minimal broth containing glycerol byproduct.
Circle, no carbon source; square, glucose; triangle, glycerol; diamond, byproduct from transesterification; star, byproduct from column.
Contentration of carbon source was .2%.
Byproduct was pH adjusted and sterilized.
Incubation at 25°C.

Glucose detection

$C_6H_{12}O_6$ --- Glucose Oxidase ---► $C_6H_{12}O_7 + H_2O_2$

H_2O_2 + DH_2 --- Peroxidase ---► D (Green) + $2H_2O$

Alcohol detection

CH_3CH_2OH --- Alcohol Oxidase ---► $CH_3C(H) = O + H_2O_2$

H_2O_2 + DH_2 --- Peroxidase ---► D (Blue) + $2H_2O$

Rapid solid phase chemistry enzyme based assays. Above, glucose oxidase oxidizes glucose to gluconic acid and peroxide – hydrogen peroxide reacts with a dye to give a green color (Precision Labs, Inc.); below, alcohol oxidase converts alcohol to an aldehyde and peroxide - hydrogen peroxide reacts with a dye to give a blue color (AlcoScreen) .

Glucose and alcohol in minimal broth cultures

		Glucose		Alcohol	
Strain	Carbon source	Control	Experiment	Control	Experiment
E. coli K12	Glucose	+	-	-	+
	Pure glycerol	-	-	-	+
K. pneum.	Glucose	+	-	-	-
	Pure glycerol	-	-	-	-

Control, uninoculated minimal broth.
Experiment, culture in minimal broth.
+, Test substrate present.
-, Test substrate absent.
The pH of cultures was 7 – 8.
Incubation at 25°C for 2 weeks.
Glycerol byproduct showed a positive test for alcohol.

COMMENTS

- *E. coli* and *K. pneumoniae* demonstrated ability to utilize glycerol byproduct as a carbon source.
- High turbidity of uninoculated control broth containing byproduct from transesterification may have decreased accuracy of measuring optical density of cultures.
- Cultures grown here were incubated with exposure to air. *E. coli* and *K. pneumoniae* can grow aerobically or anaerobically – they are facultative. It is possible that anaerobic incubation could alter the results.
- Undergraduate students assisting with the project received a valuable educational experience in laboratory investigations.
- This work helped with development of laboratory activities dealing with biofuel for undergraduate microbiology.
- The glycerol waste byproduct from biodiesel plants might eventually be used by industry to make additional products.

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