



Respiratory System of Rats Exposed to Pollutants arising out of Heating Residual Glycerol

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Abstract

Biofuels, such as biodiesel are renewable alternatives and environmentally safe fossil fuels. In the production of biodiesel, about 10% of the total volume is residual glycerol. This residual glycerol has impurities that prevent their direct use. Thus, it is important to search for alternatives to the direct use of such environmental liabilities. His direct combustion appears as a very promising route. Among the steps for using the residual glycerol as a fuel, no heating, with potential to generate pollutants whose effects on health are poorly investigated. This paper presents to study the effects of vapors from the residual glycerol heating in the respiratory system of rats. The animals were exposed to glycerol heating gas for 5 hours. As a result, an increase in the parameters of tissue elastance (H) and tissue resistance (G) of animals exposed to gases. Our results demonstrate that, despite the short period of exposure, there are changes in parameters related to lung tissue (G, H and C_{ST}), proving the harmful effect on the respiratory system of mice exposed to pollutants from the residual glycerol heating. This study can be used as reference works that intend to use the residual glycerol as fuel furnaces or boilers.

Keywords: Biodiesel; Glycerol waste; Pollution; Respiratory system

Introduction

The search for alternative energy sources and sustainable processes in order to reduce environmental pollution and global warming, has spurred the global biofuels market [1]. Biodiesel, represent a renewable and environmentally safe alternative to use of fossil fuels. As the world biodiesel production increases, while a growing concern with the co-products associated with the production of biodiesel chain, particularly with impacts on the environment and health. Special attention is being given to the abundance of residual glycerol produced. For each gallon biodiesel is cogenerated 100 mL of residual glycerol [2].

The residual glycerol in purified or glycerin form has numerous industrial applications (additives for the food industry, chemicals and pharmaceuticals), but the residual glycerol cogeneration in the biodiesel production process contains impurities, such as alcohol, water, catalyst and small concentrations of contaminants such as proteins or soaps, depending on the raw material. Purification of the residual glycerol is expensive, however some studies have been developed for the direct use of its raw form: glycerol conversion to acrolein in the presence of catalysts [3,4], production hydrogen using the residual glycerol as substrate [5], steam reforming [6] and combustion of the residual glycerol for energy use [7,8]. Among these studies, combustion is classified as one of the most promising routes in the disposal of this environmental liability due to the possibility of their direct application on a large scale. Faced with this problem, searching for viable solutions for the utilization of residual glycerol as fuel, increased knowledge of the effects of their exhaust gases to health is necessary.

One concern in using the residual glycerol as a fuel, is in awe of their toxic emissions, mainly acrolein. Acrolein is known as the product of thermal decomposition of glycerol when it is heated above 280°C. U.S. Department of labor occupational safety and health administration reports [9], suggest as permissible limit weighted to the environmental concentration of acrolein the value of 0.1 ppm for eight hours of exposure. According to criteria of the american industrial hygiene association [10] levels from 0.15 ppm for one hour are not recommended without risk of irreversible damage to human health.

Thus, among the steps for using the residual glycerol as a fuel, there is its heating, with potential to generate pollutants whose effects on the respiratory system are poorly investigated. Thus, it becomes necessary analysis of the effects caused by the residual pollutants from glycerol heating in lung function.

Materials and Methods

Animals

All procedures were previously approved by the ethics committee for animal use (CEUA-UECE). We used 16 albino rats, *Rattus norvegicus*, Wistar, males, with a body mass of 200 ± 50 g, divided into two groups. A group exposed to the vapors from the heating glycerol (n=8), called G group, and the other exposed to exhaust gases from the heating saline (n=8), called C group.

Experimental protocol

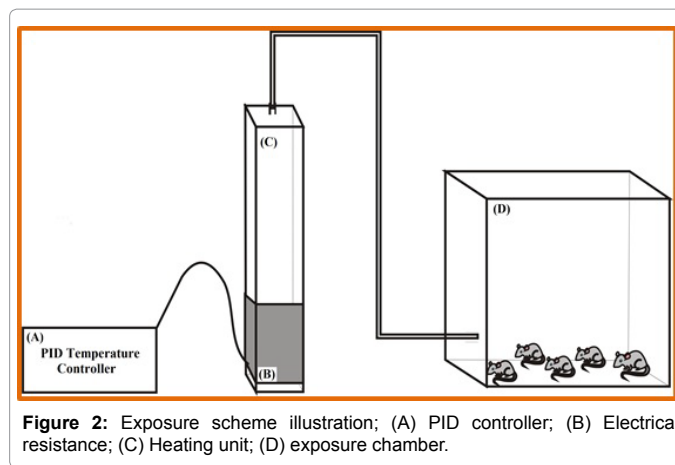
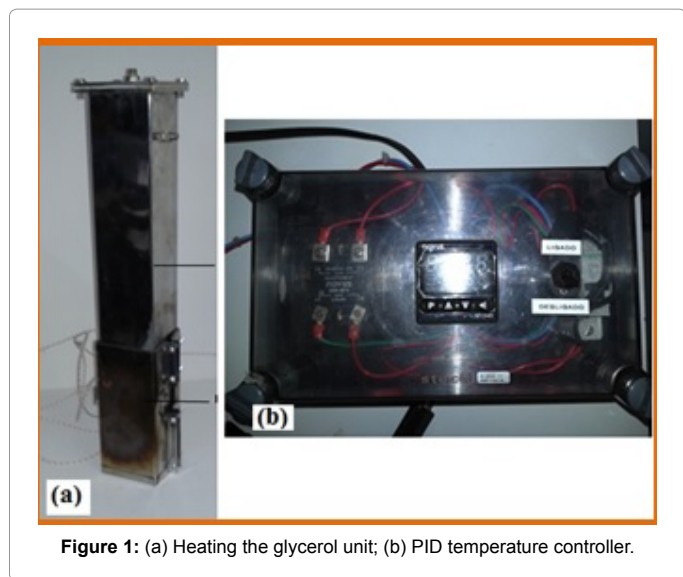
The residual glycerol used in this study comes from the biodiesel plant in Quixadá (Ceará-Brazil) derived from oil process in the ratio of 20% cotton and 80% soy. For the generation of pollutants from the residual glycerol heating, was developed a heating unit consisting of stainless steel (Figure 1a). It has long and 60 cm square base 8 cm. A resistor 2000 W was involved in the inner part of the heating unit. To have control of the residual glycerol temperature during the process,

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a circuit was developed using a PID controller (proportional-integral-derivative) temperature (Figure 1b).

The exposure protocol, both groups had duration of 5 hours. For display of glycerol group, 500 mL glycerol was added to the residual heating unit and its average temperature was maintained at 280°C. Additional volumes of 50 mL were placed with intervals of 20 minutes in the tentative heating unit to keep the initial volume of residual glycerol. For display in the control group, 500 mL of saline was added to the heating unit and its temperature was maintained at 90°C. Additional 50 mL portions, at 20 minute intervals, were also added to the heating unit in the maintenance tentative initial volume (Figure 1).

During the exposure period in both groups, the animals were accommodated in a test chamber measuring 387 mm high, 390 mm wide and 420 mm deep with a volume of about 63.5 L. Figure 2 illustrates the animal exposure scheme (Figure 2).

After the exposure period in both groups, the animals were anesthetized with sodium pentobarbital (50 mg/kg Hypnol[®] 3% Syntect, Brazil) intraperitoneally, and subjected to tracheotomy for the introduction of a cannula 14G (Eastern medikit LTD) which was set to the trachea. The cannula was then connected to a respirator for small animals (Scirec-flexVent[®]) controlled by computer. The animals were ventilated at baseline standards at a frequency of 90 breaths/min, a tidal volume of 10 mL/kg, with maximum pressure of 30 cm H₂O, and a positive end-expiratory pressure (PEEP) of 3 cm H₂O.

For the variables of lung function were collected reliably, it is necessary that the animal is paralyzed for no interference from the activity of your respiratory muscles. Therefore, after the start of ventilation, it proceeded with pancuronium bromide (0.5 mL/kg, Pancuron[®], Cristália, Brazil), intraperitoneally.

Initially we perform the standardization of the mechanical conditions of the respiratory system with application of two deep inflations (IPs) with maximum pressure of 30 cm H₂O and 6s duration. To reduce the effect caused by broncoprotetor IP [11], the animal was ventilated for a period of 20 minutes in basal patterns. Soon after, the impedance of the respiratory system (Z_{rs}) was measured by forced oscillation technique sequentially in 30s intervals for 6 minutes.

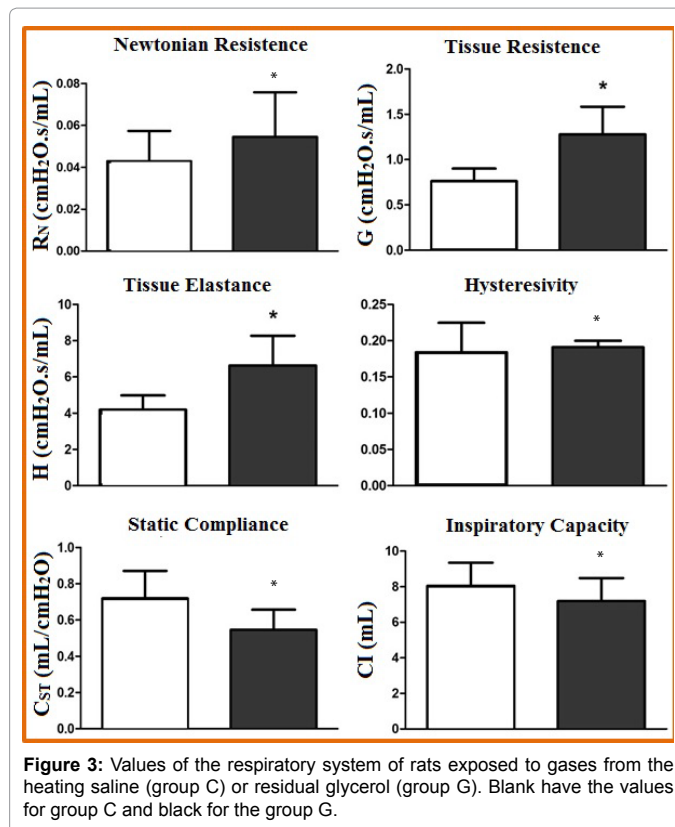


Figure 3: Values of the respiratory system of rats exposed to gases from the heating saline (group C) or residual glycerol (group G). Blank have the values for group C and black for the group G.

The Z_{rs} was determined by measuring the volume displacement and cylinder pressure piston blower as disorders 3s oscillatory volume were delivered to the airways. These disorders were made using 13 sinusoidal waves superimposed with variations of amplitude and frequency (1 to 20.5 Hz). The frequencies were fixed at values mutually conditioned to reduce the harmonic distortion that can occur in nonlinear systems [12]. Before the protocol was obtained signals dynamic calibration required to correct the physical characteristics of the mechanical ventilator in subsequent measurements Z_{rs} . The Z_{rs} was determined by Fourier transform of the volume signals of the fan piston and the cylinder pressure, as described previously [12]. The Z_{rs} was calculated according to the model,

$$Z_{rs} = R_N + I(2\pi f)i + \frac{G - Hi}{(2\pi f)^\alpha}$$

Where,

$$\dot{a} = \frac{2}{\pi} \tan^{-1} \left(\frac{H}{G} \right)$$

Where, R_N is Newtonian resistance which represents the resistance of the central airways, i is $\sqrt{-1}$, f is the frequency (Hz), I is the inertance of the airways, and G and H respectively characterized dissipative properties and elastic lung tissue [12]. Another important characteristic of the constant phase model, with respect to the ratio G/H , known as histeresividade (η). Soon after, it was held two curves PV (pressure-volume) for analysis of static compliance (C_{ST}) and estimated inspiratory capacity (IC).

Statistical analysis

Results are presented as mean \pm standard deviation will be considered statistically different results that show the probability of occurrence of lower null hypothesis that 5% ($p < 0.05$). The data will be analyzed using t-test of student.

Results

The results of the analysis of the respiratory system of rats data are shown in Figure 3. The tissue elastance values (M), tissue resistance (G) and static compliance (C_{ST}) showed statistically significant differences between groups.

The numerical values for the variables of the respiratory system analyzed are presented in Table 1.

Discussion

Our results demonstrate that, despite the short period of exposure, there are changes in lung function of animals exposed to pollutants from the residual glycerol heating. The Newtonian resistance (R_N) has been used as a good estimate of the total resistance of the central airways [13]. We did not observe statistically significant change in R_N between the groups, though the R_N parameter only presents the estimate of the extent of the central airways and there may be changes in small airways, either by accumulation of secretion or the closure of these. Changes in R_N may be an indication of a greater narrowing or increasing the stiffness of airway smooth muscle [14].

We also observed the results characteristics of an increase in tissue stiffness with increased G and H between groups. There are several hypotheses to explain their changes, one of them is due to the

modification of the rheological properties of the tissue [13]. Another way would be through the influence that the narrowing of the airways has on these parameters, narrowing which could result in a distortion of the lung parenchyma with small airway closure, providing an effectively lower lung with an H proportionally higher [15]. The tissue resistance (G) reflects the viscous dissipation of energy in the lung tissue parameter is also changed due to the distortion of the lung parenchyma that occurs when the airways constrict [15]. Significant increases in the G and H values can be explained by the presence of mucus in the airways of small arms, leading to occlusion of these airway providing areas of atelectasis.

Pulmonary histeresividade (η) is a parameter that is associated ventilatory heterogeneities in the lung, in the lung histeresividade values groups showed no significant differences estatisticamentes. This variable is closely related to R_N and likely changes in this variable were not enough to cause a significant change in pulmonary ventilation homogeneity.

Regarding the parameters obtained by performing the curve pressure volume (PV), we observed no significant changes in static compliance parameters (C_{ST}) and estimated inspiratory capacity (IC). These variables are calculated from the equation of Salazar and Knowles, this equation is only used for setting the upper half of the expiratory branch of the PV curve. Changes in the parameters of the PV curve, are generally associated with changes in the production or distribution of surfactant in alveolar surface. The lack of change in C_{ST} and CI reflects the absence of changes in this mechanism.

Article similar to ours was conducted by Renne et al. [16], where they conducted a study of glycerol aerosol inhalation for 2 and 13 weeks, with different concentrations of glycerol (0.1, 1.93 and 3.91 mg/L for 2 weeks and 0, 0.033, 0.167 and 0.662 mg/L for 13 weeks) in rats and were mainly a result the development of squamous metaplasia of the epiglottis of the epithelium in aerosol inhalation concentration of 0.167 mg glycerol/L in the group submitted to 13 weeks of inhalation. Studies have demonstrated that mice sensitized with ovalbumin and exposed to diesel exhaust gases 12 hours a day, 5 days per week for 5 weeks had allergic inflammation and airway hyper responsiveness, and a significant increase in respiratory system resistance [17] and the intratracheal administration of diesel exhaust particles (SDP) sensitization with ovalbumin (OVA) in mice induces inflammation and hyperresponsiveness of the airways and mucus hypersecretion [18].

Conclusion

Our results show the harmful character of gases from the glycerol heating health. This study serving as a reference work that wants to use the residual glycerol as fuel. Represent a starting point for creating air quality standards in environments with these gaseous pollutants, since the amount of residual glycerol tends to increase due to the increasing production of biodiesel and studies which seek to facilitate the use effectively are extremely important.

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Measure	Group	Value	p value (t student)
Newtonian resistance (R_N) (cmH ₂ O.s/mL)	G	0.0544 \pm 0.0212	p=0.3424
	C	0.0428 \pm 0.0143	
Tissue Elastance (H) (cmH ₂ O)/mL)	G	6.629 \pm 1.642	p=0.0172*
	C	4.190 \pm 0.786	
Tissue Resistance (G) (cmH ₂ O/mL)	G	1.278 \pm 0.304	p=0.0087*
	C	0.762 \pm 0.138	
Histeresivity (η)	G	0.190 \pm 0.008	p=0.7274
	C	0.184 \pm 0.040	
Inspiratory Capacity (IC) (mL)	G	7.199 \pm 1.288	p=0.3244
	C	8.057 \pm 1.295	
Static compliance (C_{ST}) (mL/cmH ₂ O)	G	0.516 \pm 0.110	p=0.0411*
	C	0.719 \pm 0.151	

Table 1: Experimental measurements of variables related to the calculation of impedance of the respiratory system (Z_{Te}) and the PV curve. Values shown are the mean \pm standard deviation $p < 0.05$ Considered statistically significant.

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