Biogas production from anaerobic digestion of grass was evaluated in this study taking into account two harvesting machines, a Disc-mower and an Excortior, under diverse operating conditions. In addition, the application of generated biogas either in a Combined Heat and Power (CHP) plant for thermal and electrical energy production or as transportation fuel after upgrading (BGU) process was evaluated. Consequential Life Cycle Assessment (CLCA) with long term marginal data was employed. Lab-scale data as well as those obtained from the ecoinvent database were used to compile life cycle inventory data. The system boundary of the present study covered harvesting operation of grass, baling, transportation of bales, anaerobic digestion, use of digestate on farmlands, and downstream processes for biogas usage. Additionally, the system boundary was expanded to take into account the effect of substituting grass with straw in animal feeds. The results demonstrated that the environmental performance of grass-based biogas plants were highly dependent on selected downstream strategies. Furthermore, it was evident that mono-digestion of grass would not guarantee a long-term sustainable renewable energy system. Based on the results obtained, Excortior at driving speed of 7.5 km/ha had the best environmental performance in all damage categories, i.e., “Human health”, “Ecosystem quality”, “Climate change”, and “Resources”. CHP had a greater environmental performance than water scrubbing BGU for the downstream strategies taken into account. The results from the sensitivity analysis proved that a specific methane yield lower than 329 mLCH4/gVS cannot ensure the achievement of an eco-friendly energy system from grass-based biogas plants.